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## EARLY LESIONS OF RHEUMATIC ENDOCARDITIS \*

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BOSTON

*When we look at these questions (of cure, of unexplained fever) from the aspect of morbid anatomy we can well understand how much further investigation is required to answer them, and how much our difficulties are increased by the great rarity of fatal rheumatic heart disease in the very earliest phases of the illness in man.—Poynton.<sup>1</sup>*

The basis for this thesis is the opportunity furnished me, as medical examiner of Suffolk County, to study the early phases of rheumatic endocarditis in persons who came to violent deaths while in apparent full health, or who died suddenly without hospitalization, and on whose cardiac valves were found—often as casual discoveries, unrelated to the cause of death—the early lesions of rheumatic endocarditis.

The hospital pathologist deals largely with the end-results of rheumatism, in which the disease itself, the inefficiency of tissues due to its lesions, or superinfections, terminal or otherwise, lead to the fatal outcome. The literature is rich in contributions based on this material, but lacking in those based on the initial or early lesions.

There is presented in the following paper a description of an unusual tissue reaction in the form of a cell palisade along the contact edges of the cardiac valves, which appears to be a specific response of allergic tissue to an infection. In its earliest phase it has not been described in the literature. The absence of an acute inflammatory reaction in the tissues of the valve, and the apparent efficiency of the defense afforded to the underlying tissues in the presence of infecting organisms on the surface, are striking. The process is diffuse, covering the whole of the contact edge of the valve.

The probable method of evolution of the verruca, characteristic of rheumatic endocarditis, as the result of focal injury to the cells of this palisade, is illustrated. Furthermore, the diffuse formation of scar tissue directly from the cells of the palisade explains the diffuse scarring, apart from the verrucae, met with in rheumatic endocarditis.

The endocardial lesions of rheumatism have been given less attention since Aschoff's description of the myocardial nodule. And yet these

\* Submitted for publication, April 16, 1931.

\* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Poynton, F. J.: *Lancet* 2:536, 1928.

lesions are the most constant and grossly the most characteristic evidences of rheumatic infection. Poynton and Paine<sup>2</sup> found mitral lesions in 149 of 150 hearts of children dying from rheumatic morbus cordis, while in the literature on the Aschoff nodule Clawson<sup>3</sup> found records of 250 hearts with rheumatic endocarditis or chorea, examined for Aschoff nodules, in 26 per cent of which no nodules were discovered.

Reasons for believing that the cell palisade is an early reaction of rheumatic infection are discussed. If, as appears, this lesion is an early and characteristic response to rheumatic infection, it supplies a desideratum for experimental effort more definite than the nondescript valve lesions which have been produced so far.

Most of the textbook illustrations of rheumatic lesions of the cardiac valves deal with advanced processes the evolution of which is ignored. A rational explanation of the origin of these lesions, particularly the vegetations, seems to be evident from the material presented here.

#### THE LESIONS

The principal material for this study was obtained from three patients: the earliest phase from an apparently healthy boy of 6 years, whose death was due to violence, with an endocardial lesion of the mitral valve before verruca formation; an intermediate phase from a girl of 18 years, who had mitral stenosis for eight years before her death, with the endocardial lesion on the tricuspid valve, associated with verrucae formation in various stages; a late phase from a man, aged 51, who had an old, but not advanced, mitral process, with the lesion on the mitral valve, and showing pseudoverrucae due to thrombus formation.

CASE 1.—J. B., 6 years of age, was struck by an automobile and killed practically instantly. Inquiry at his home disclosed that except for an attack of measles when he was 5 years of age he had had no serious illnesses. He was an ordinary boy, not particularly robust, and had complained at times of a sore throat with an occasional head cold, the last attack having occurred several weeks before his death. His recovery from this condition had been apparently complete. The postmortem examination, apart from the evidences of violence, which included a fractured skull, disclosed no gross or microscopic changes except those found in the heart and tonsils.

The heart was of normal size. Along the whole of the contact edge of the mitral valve was a delicate yellowish band, as though one had dipped a brush in fibrin and painted a narrow girdle, in general about 0.2 cm. in width, but varying up to 0.5 cm. in places, along the whole circumference. There were no vegetations at any point. Smears from this layer showed gram-positive diplococci.

The tonsils were small with scarred surface and small crypt openings. Section disclosed bottle crypts, one in each tonsil containing puriform material. Smears

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2. Poynton, F. J., and Paine, A.: *Researches on Rheumatism*, New York, The Macmillan Company, 1914, p. 34.

3. Clawson, B. J.: The Aschoff Nodule, *Arch. Path.* 3:664, 1929.

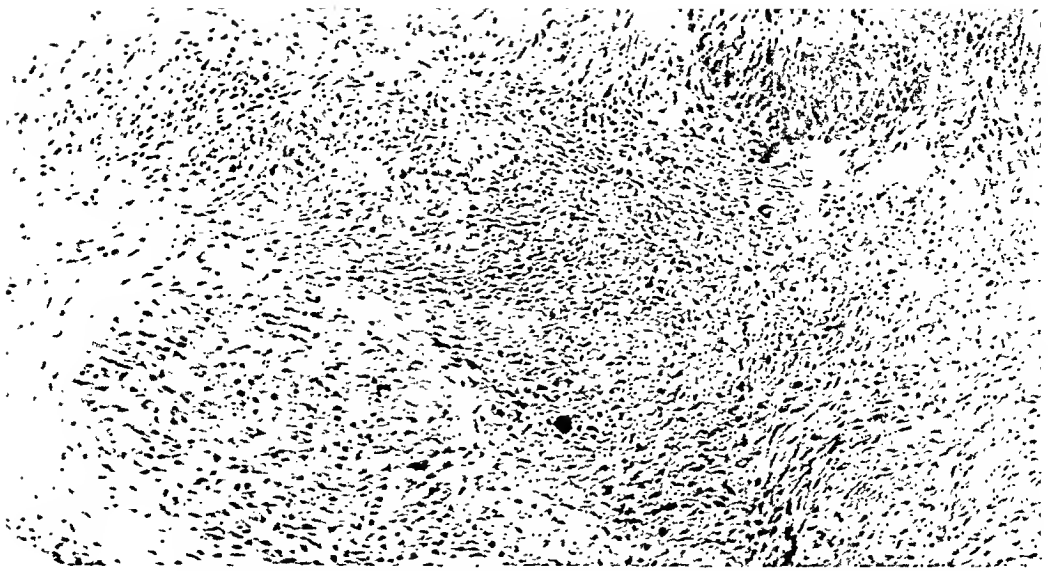


Fig. 1.—The cell palisade on the surface of the mitral valve;  $\times 106$ .

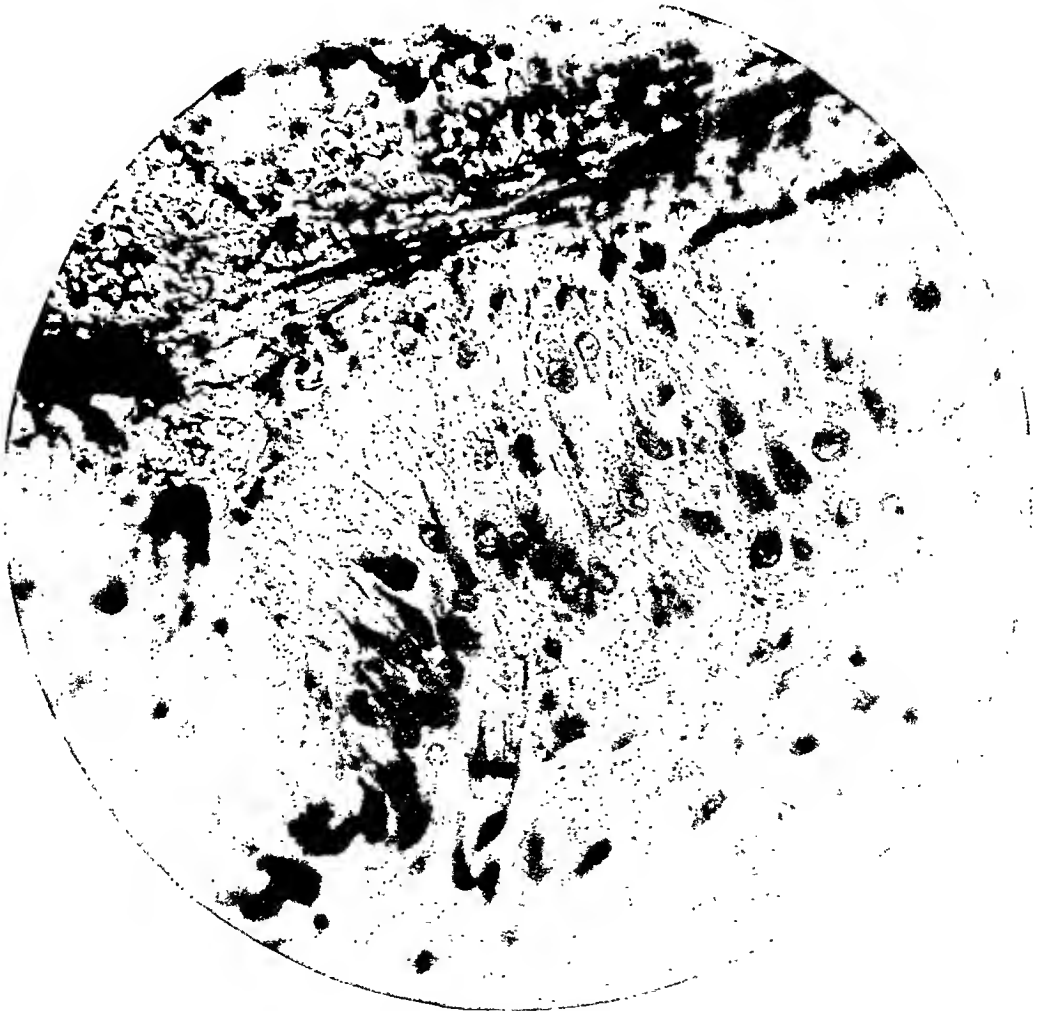


Fig. 2 (case 1).—Group of cells of the palisade, with details of the exudate;  $\times 485$ .



from this material showed pus with predominating gram-positive diplococci and short-chained streptococci. Cultures from the cardiac valve produced a growth of *Streptococcus viridans* in purity. From one tonsil an almost pure culture of *S. viridans* was obtained.

Microscopically the valve showed recent and older lesions. The contact edge of the valve was covered by a continuous layer of cells set on edge at right angles to the valve surface. These cells were elongated with ovoid or pyriform cell bodies attached to the subendocardial tissue by their bases and with oval vesicular nuclei. The cell bodies were prolonged by delicate filiform extensions that terminated in oval bulbous ends at the surface. Between the filiform extensions were delicate fibrillae and interfibrillar spaces. In places the cells were grouped in series with the cell bodies accommodating themselves to one another in an arrangement corresponding somewhat to that found in the stratified columnar epithelium of the lower larynx or trachea. Here and there in this layer occurred free cells, oval or irregular, without obvious processes, and with oval or indented vesicular nuclei. Where edema was present there was wide separation of the cells and particularly of their processes. Mitotic figures were rare. The whole effect of this layer was that of an orderly palisade of cells guarding the injured valve surface.

The surface of this palisade was covered by a thin layer of red cells, with fibrin, not compact, but in a rather coarse meshwork. An almost continuous single row of polymorphonuclear leukocytes lay along the cell processes beneath the fibrinous layer. Gram-positive diplococci were scattered through the meshes of the fibrin and in rare masses here and there.

The tissues beneath the palisade were slightly edematous and infiltrated with cells, some of the type of lymphoid and plasma cells, but for the most part with large, oval vesicular nuclei and having the character of histiocytes. There was also evidence of an increase in the fixed fibroblasts. The tissues about the contact edge were avascular, save that a vessel appeared beneath the portion of the lesion nearest the base of the valve.

This was the standard picture of this portion of the valular lesion. In places, however, the ends of the cell processes of the palisaded cells showed degeneration, and took the eosin stain heavily, appearing as a continuous beaded line. This effect seems to be the result of injury. Elsewhere, apparently due to more serious injury, the affected cell processes were fused into a dense hyaline material, staining even more deeply with eosin. Where the damage was still greater, associated with clumps of bacteria, the orderly grouping of the cells was replaced by a more chaotic arrangement, and regions of necrosis of the surface layer about bacterial masses were found.

In older lesions of the valve, there was definite vascularization of that portion of the valve leaflet nearest its base in the form of young vessels with cellular walls.

Portions of the valve surface showed over considerable areas chaotic disarrangement of the cells of the palisade, many of which were necrotic in places.

Over a large area in some sections alongside of the palisaded area the surface was covered by a relatively acellular connective tissue presenting in general a smooth surface, but covered intimately by a thin layer of necrotic material. In this portion of the valve there were found just beneath the surface a few larger collections of bacteria, which lay in oval or angular spaces. In these regions the surface layer was necrotic, the necrosis extending about the bacterial masses, so that it was difficult to determine whether the spaces containing the organisms were

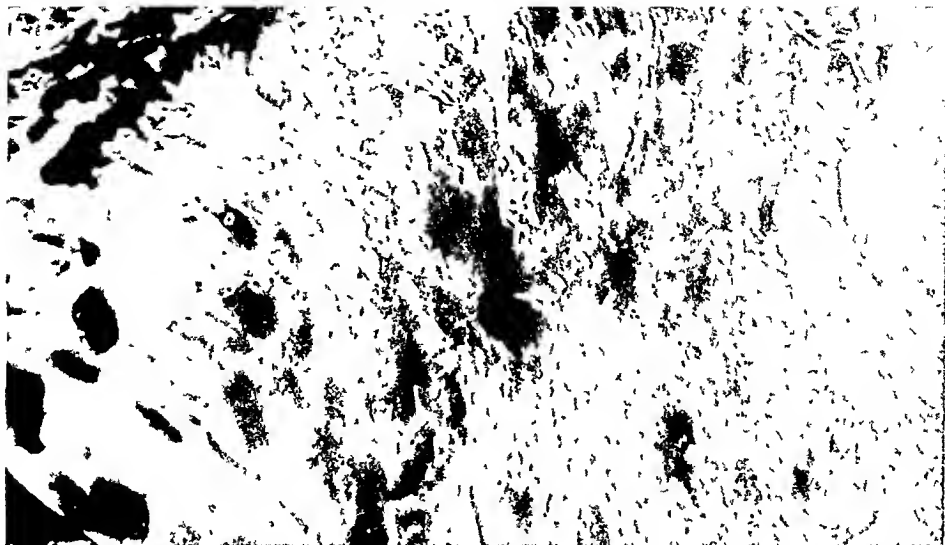


Fig. 3 (case 1).—The beaded necrotic end-plates of the cells of the palisade. Note the free cells—histiocytes;  $\times 650$ .

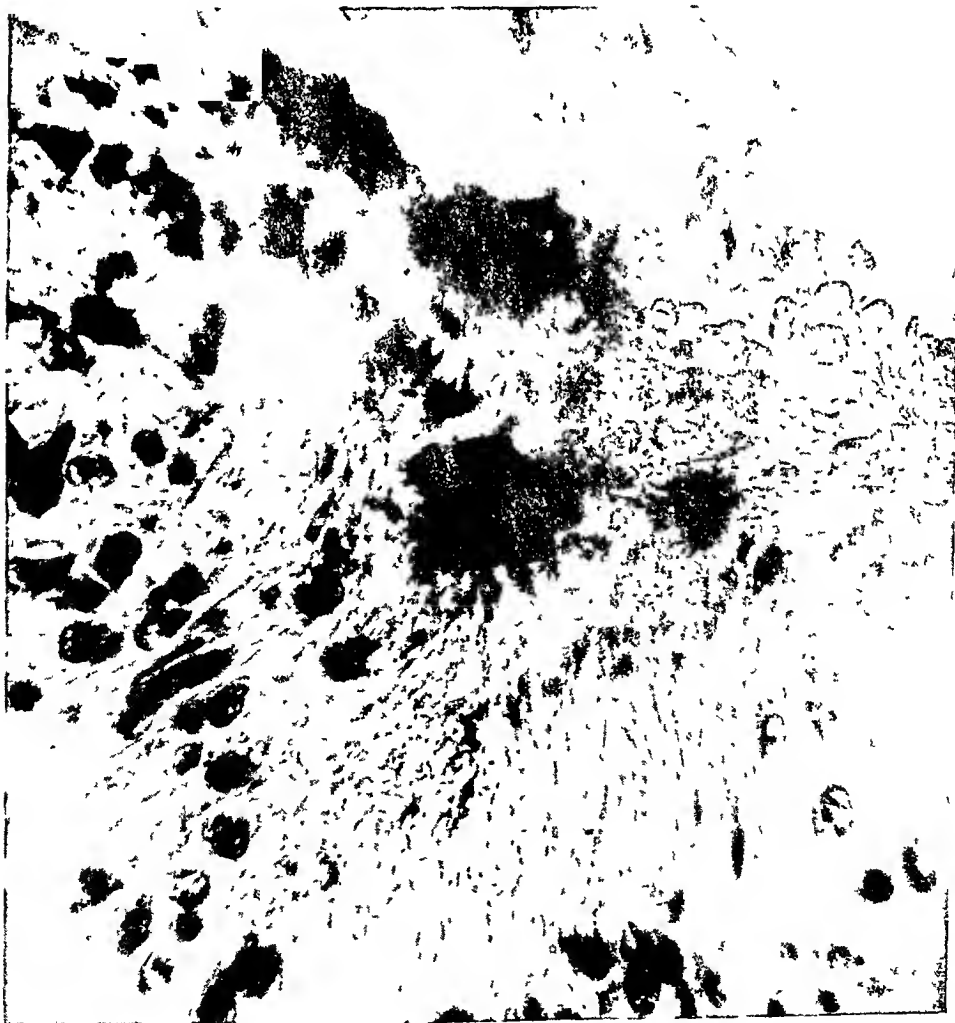


Fig. 4 (case 1).—An octopus-like hyaline necrotic mass. The projections are the necrotic processes of cells, which show disorganization below;  $\times 650$ .



Fig. 5 (case 1).—Bacteria in masses in spaces in the necrotic surface layer of the valve;  $\times 830$ ; Gram-Weigert stain.

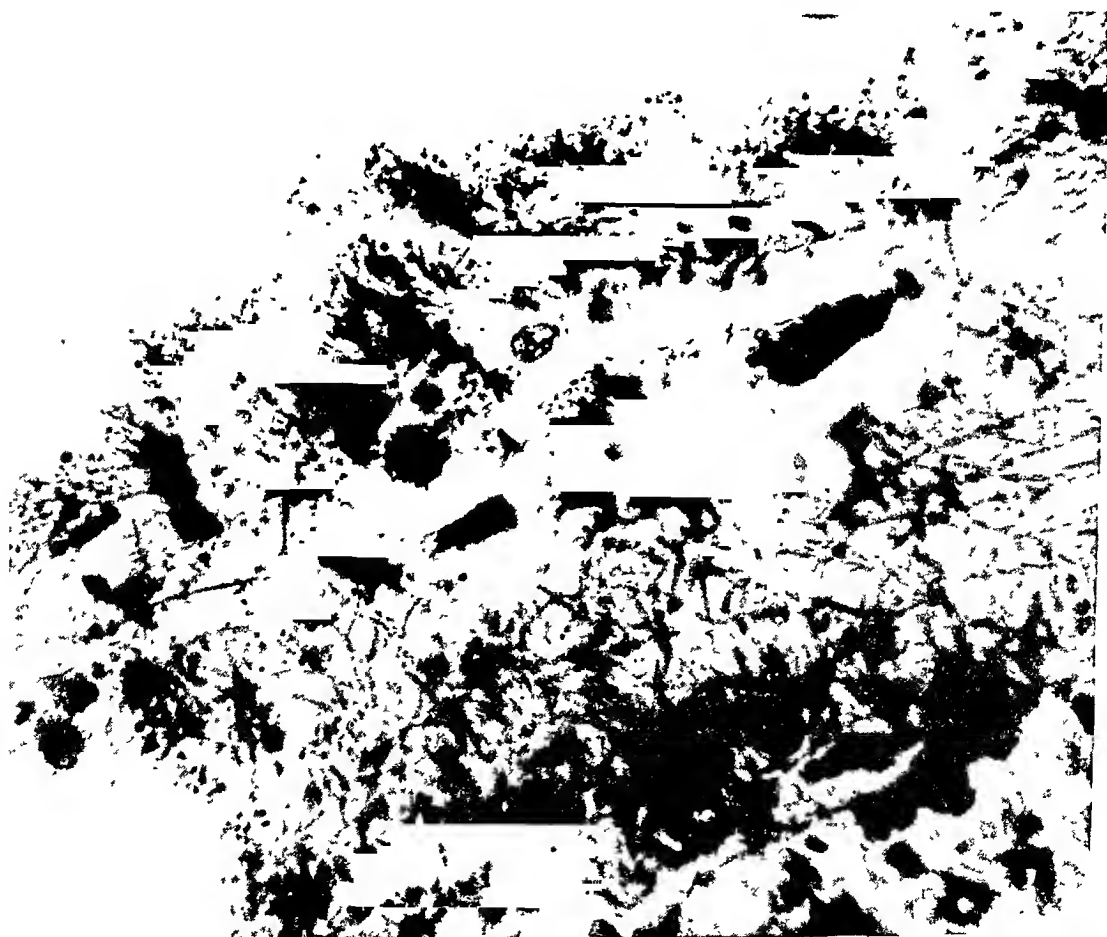


Fig. 6 (case 1).—Bacteria as diplococci or in small masses scattered in the exudate on the surface;  $\times 1,115$ ; Gram-Weigert stain.

vessels. Their appearance suggested it, but no vessels were found in the surrounding valve tissue. In the necrotic area some of the histiocytes exhibited pyknotic nuclei, and the cell bodies took a deep stain. Lymphoid cells within the necrotic region still took the nuclear stain. No acute inflammatory reaction appeared about these necrotic regions.

In the palisaded area bacterial masses were found lying within folds of the valve edge, which were overhung by the surface layer in such manner that the spaces containing the organisms could well have been converted into a semblance of the picture described in the foregoing paragraph when necrosis took place.

The tissues of the body of the valve showed edema with infiltration by histiocytes and by lymphoid and plasma cells. There was also an apparent increase in fibroblasts. Striking was the absence of polymorphonuclear leukocytes in all parts of the valve tissues. They were found only in the deeper layers of the exudate on the valve surface.

CASE 2.—D. H., a girl, was admitted to the Boston City Hospital May 29, 1923, at the age of 11, with rheumatic fever and subacute endocarditis of the mitral valve. At the age of 1½ years she had diphtheria and at the age of 7 years whooping cough. There was no history of tonsillitis or of growing pains. She had been active physically and bright mentally. One month before entry she had been in bed for a day or two with a cold in the head and a sore throat. She improved promptly and returned to school. She continued at school throughout the week preceding her admission to the hospital, though she had recurring attacks of sharp pain in the upper part of the abdomen, lasting about an hour and occurring often twice a day. On Sunday, May 28, the pain was more severe and kept her awake until the middle of the night, being finally relieved by a hot water bottle. She was admitted to the hospital on the following morning. She complained of pain in the left great toe.

Physical examination disclosed injection in the pharynx. The tonsils were small, with injection on the surface, which showed no exudate. There were a few palpable anterior and posterior cervical glands. There was marked tenderness over both angles of the jaw.

The heart was not enlarged. The apical beat was just below the nipple. There was no precordial impulse. The right border was at the right sternal margin; the left was 7.5 cm. to the left of the midsternal line. Over the mitral and pulmonic areas and along the left border of the sternum there was a soft systolic murmur, not transmitted to the axilla. The heart sounds were clear and distinct. The rate and rhythm were regular. The right metatarsophalangeal joint was swollen and tender.

Two days after the patient's entrance a rough systolic and mid-diastolic murmur was heard at the apex. The systolic murmur was heard over the aortic area. At times a diastolic murmur, singing in quality, was heard to the left of the sternum.

Pain developed in the right upper thigh, left ankle and right knee, but was controlled by salicylates and oil of wintergreen dressings.

The temperature varied from 99.8 to 102 F. during the first three days of the patient's hospitalization and then dropped to normal. A second rise in temperature occurred between the fourteenth and eighteenth days. White blood cells were 17,000 on the second day, dropping to 6,400 on the twelfth day. The patient was discharged from the hospital on the forty-eighth day with a mid-diastolic murmur heard in the third left interspace.

There is no further medical record save that a district physician of the Boston Dispensary was called Aug. 27, 1929, when the patient was suffering from a cold. He made a diagnosis of rheumatic heart disease: mitral stenosis.

The girl worked as a coffee packer up to the day before her death, which occurred Oct. 23, 1930. On that evening she attended a dance, as was her custom at intervals. She was seized with chills and dyspnea. The next morning a local doctor was called, who found her flushed, dyspneic and cyanotic, with dulness over the anterior part of the chest. He made a diagnosis of lobar pneumonia and ordered hospitalization. She was pronounced dead on arrival at the hospital.

Postmortem examination revealed a well developed, but rather poorly nourished, girl. The length of the body was 5 feet, 2 inches (157.5 cm.); the weight was 92 pounds (41.7 Kg.). There was no clubbing of the fingers; there was some cyanosis of the lips, but only moderate lividity of the back. There was white froth in the mouth.

The heart weighed 255 Gm. The mitral valve appeared as a somewhat thickened, opaque membrane showing no distinction between anterior or posterior flaps and with an almost central oval opening, 1.5 by 0.7 cm. The edges of the orifice were smooth, without suggestion of vegetations. The left auricle and ventricle were moderately dilated. The endocardium lining the left auricle was thickened and opaque, with some flattening of the irregularities of the wall. The aortic valves showed slight old thickening about the corpora arantii.

On the tricuspid valve was a discontinuous series of verrucae with an intervening slightly raised, roughened, yellowish layer along the whole of the contact edge. At the base of the posterior or septal flap there was valvulitis with fibrous adhesion of the proximal portion to the underlying muscle. Along the contact edge of this flap was a series of flat, plaquelike granulations.

There was massive edema of both lungs. The other organs showed only general passive hyperemia.

Microscopic examination of the tricuspid valve revealed along the contact edge collections of small verrucae, as many as four in series appearing in some sections made at right angles to the edge. Between the verrucae, and in places where there were no verrucae, a palisade of cells, irregular in places, and set at right angles to the edge, was found. This palisade was also present on the surface of most of the verrucae. Unlike the lesion described in the heart showing the first phase of this stage, the surface of the palisade was covered only by a thin layer of hyaline necrotic material, or masses of platelets with occasional clumps of polymorphonuclear leukocytes, or by a thin layer of red blood corpuscles evidently recently deposited. Examination revealed no bacteria.

The palisade was less perfect than that in case 1. In places there was heavy infiltration of this layer with histiocytes. In other places there was edema with degeneration and evident loss of a large portion of the cells. The picture in these degenerating regions approached that found in case 3.

The valve tissues showed in places infiltration by lymphoid cells, with a rare polymorphonuclear leukocyte, and histiocytes, occasionally in mitosis, were found in small numbers through the tissues adjacent to the contact edge.

There was evidence of an older process of repair in a diffuse increase of connective tissue, in vascularization of the valve beneath the verrucae, and in extensive vascularization of one of the larger verrucae. In one region there was a definite small focus of granulation tissue on the surface of the valve.

Beneath the cell palisade in places was a loose, somewhat edematous young connective tissue in which it was possible to make out the transformation of the cells of the palisade into connective tissue by the formation of intercellular fibrillae.



Fig. 7 (case 2).—Cell palisade covered with platelets;  $\times 80$ .



Fig. 8 (case 2).—Detail of cell palisade in an edematous portion. Note the bulbous end-swellings of the cells in the middle region;  $\times 400$ .

Aschoff nodules were found with little difficulty.

The pulmonary process was one of massive edema. No acute inflammatory reaction was present. The alveolar vessels showed injection, with some thickening of the alveolar walls and histiocytic infiltration. In the alveoli were collections of large cells containing hemosiderin, averaging perhaps three per alveolus. Many alveoli contained none, and a few alveoli were crowded with massed cells. There was no infiltration by polymorphonuclears or formation of fibrin.

In the other organs was present only a moderate degree of chronic passive hyperemia.

CASE 3.—M. T., a chronic drunkard, 51 years old, was known to have fallen on the street several days before his admission to the Boston City Hospital, and was found unconscious by his landlady after a continued spree. He died three



Fig. 9 (case 2).—Formation of connective tissue from the cell palisade;  $\times 200$ .

days after entrance, without recovering consciousness and without exhibiting any localizing cerebral symptoms.

The postmortem examination disclosed a scalp wound without fracture of the skull. There were present chronic pachymeningitis and leptomenigitis. The cerebral vessels were markedly sclerotic, and section of the brain revealed series of small foci of softening in the silent area characteristic of arteriosclerotic disease with thrombosis. There was massive bilateral bronchopneumonia. In the spleen were a series of recent hemorrhagic infarcts.

The heart weighed 410 Gm. The anterior flap of the mitral valve was thickened along the contact edge, and there was some shortening with thickening of the chordae near their attachments to the midportion of this flap. Attached to the midregion of this flap was a vegetation-like mass of clot, and a smaller mass lay along the contact edge to the left of the midline. A similar mass,

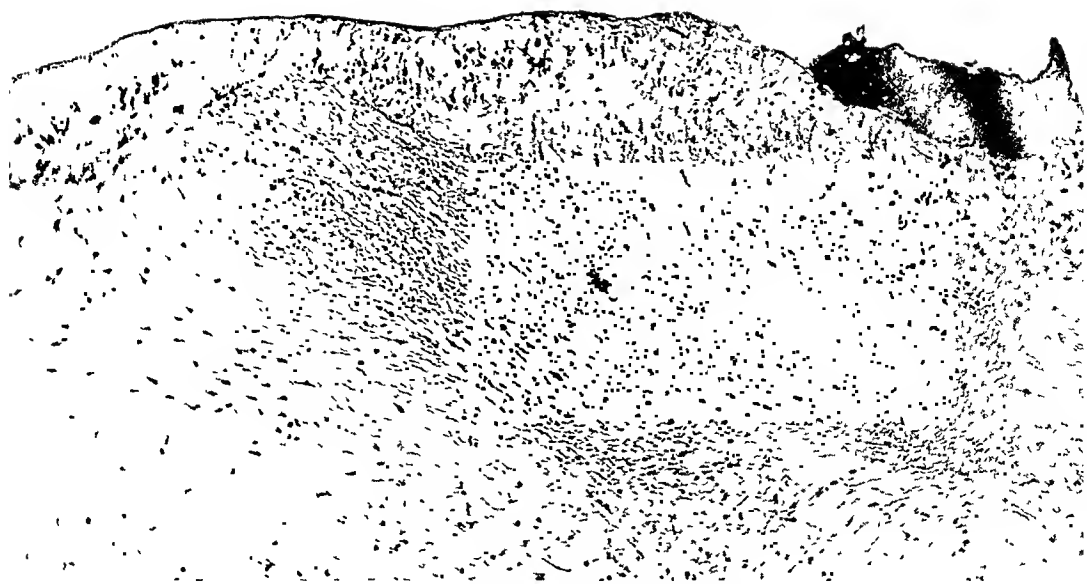


Fig. 10 (case 3).—Remains of degenerating cell palisade;  $\times 80$ .

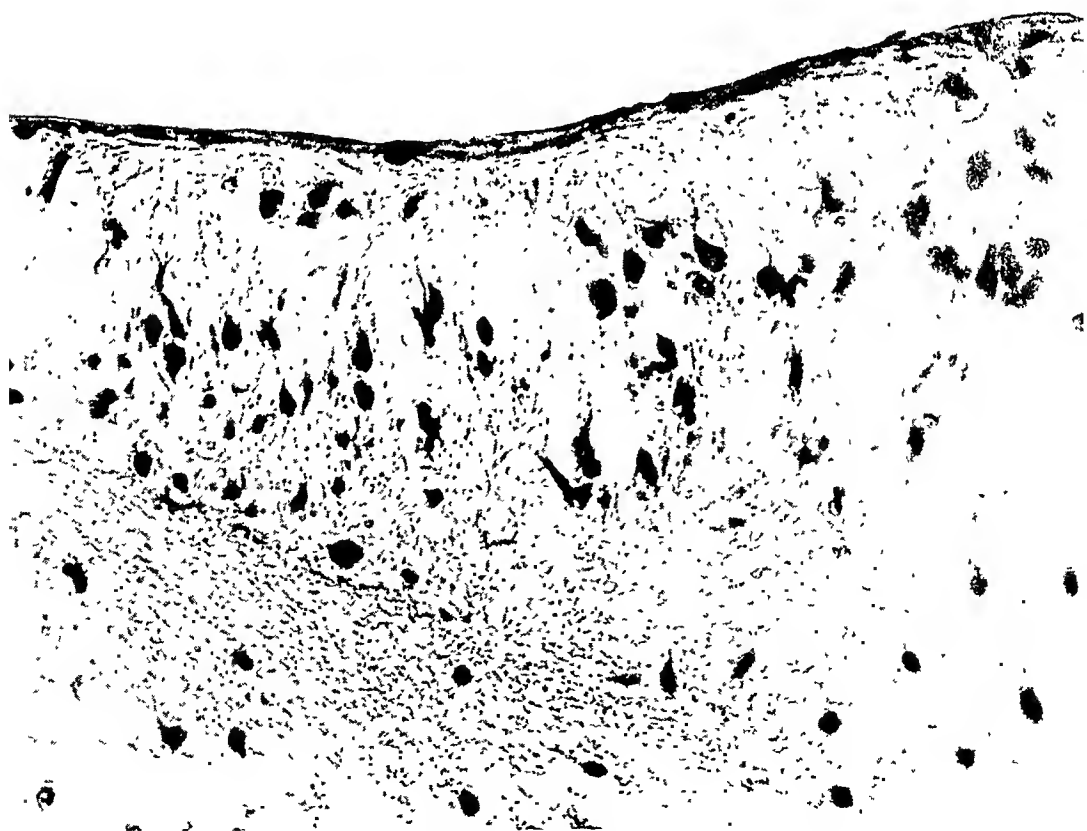


Fig. 11 (case 3).—Detail of degenerating cell palisade;  $\times 400$ .



elongated laterally, was present in the midportion of the posterior flap along the contact edge. There was marked dilatation of the cavity of the left auricle, with thickening of the endocardium and flattening of the folds.

Microscopically the pseudoverrucae on the valve surface were made up of clotted blood, rich in fibrin and platelets, which was attached to portions of the surface denuded of endocardium and made up necrotic, dense, relatively acellular connective tissue. Alongside of the necrotic region the valve surface was covered by a layer of cells showing high-grade degeneration. These cells were set at right angles to the surface. The cell bodies were shrunk and in part fragmented, and the intervening space was occupied by delicate fibrillar material, the general direction of which was at right angles to the surface. Many of the cells appeared as almost naked nuclei, having lost most of their cytoplasm, fragments of which lay in connection with, or slightly separated from, the perinuclear mass.

The whole picture of this third phase indicated high-grade degeneration of a palisade-like layer of cells, proceeding to atrophy and necrosis without shrinkage in the size of the layer which the cells had originally made up. The sites which they occupied had come to be filled with an edematous, loose-meshed fibrillar structure with a distinct suggestion of mucoid degeneration. It is this phase that has been described by Königer<sup>4</sup> in five cases of lesions of the mitral valve and discussed by Ribbert.<sup>5</sup>

#### COMMENT

*The Cell Palisade.*—In case 1, in which the palisade was seen in greatest perfection, certain conclusions are apparently justified. There can be little question that this was a reaction of defense. The orderly arrangement of the cells with their processes bristling toward the surface suggests that protection was being furnished against an injurious agent located on that surface. The relation of the bacteria to the cells supports this thesis. It is further indicated that the reaction arose on the surface of the valve and not from the depths. The absence of vascularization in the tissues of the valve below the palisade, save for one vessel beneath the edge of the palisade on the proximal side, is confirmatory as far as it goes.

The only acute exudative elements were found on the surface of the lesions. There were no polymorphonuclears or fibrin in the valve tissues. The exudate must therefore have been formed from the blood flowing over the surface.

In acute inflammation the mobilization of reserve polymorphonuclear leukocytes from the marrow, and their concentration at the locus of injury, have been likened to the mobilization of an army and its transportation to the seat of war. It is a general defensive movement. The reaction illustrated by this palisade is a local defensive effort, analogous to the military procedure in trench warfare.

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4. Königer, H.: *Histologische Untersuchungen über Endocarditis*, Arb. a. d. path. Inst. zu Leipsig, 1903-1908, nos. 1-5, p. 40.

5. Ribbert, H.: *Die Erkrankungen des Endokards*, in Henke and Lubarsch: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1924, vol. 2, p. 217.

Gay and his co-workers<sup>6</sup> showed that the efficient defense against streptococcal infection is not that produced by an acute inflammatory exudate, but that depending on the activities of histiocytes. The palisade formation on the cardiac valves is a more complex reaction than the relatively simple mobilization of either polymorphonuclear leukocytes or histiocytes, each of which presumably responds to simple chemotaxis. These mobile cells, when attracted to the site of injury, act as individual units, though often massed into groups. There is no interaction between individual cells. The palisade, on the other hand, is an evident cooperative reaction, with the establishment of an orderly relation between the component fixed cells and the associated histiocytes. Its appearance suggests that the effort is carried out by tissues in a state of preparedness. The evidence in all these valves of earlier infection, i. e., the indication that the active process was the result of reinfection, further suggests that this state of preparedness probably arises as the result of allergy.

In trying to connect the palisade with other tissue reactions the most obvious possible relation is to granulation tissue. Granulation tissue is due primarily to a vascular reaction. The implication that the palisade takes on its peculiar formation on the valves because these structures lack blood vessels is unsatisfactory, because in case 2 vascularization of the valve was general. In places vessels were found directly beneath regions showing almost perfect palisade formation. Alongside such a region was found one small focus of granulation tissue, with a multiplicity of new vessels exposed almost naked on the surface, in sharp contrast to the palisade surface, in which the vessels displayed less activity and did not approach the cells of the palisade.

There are three possible sources of the cells of the palisade. They may be derived from endothelial cells, but the evident destruction of the endothelium in toto over the affected region makes this highly improbable. They may arise from histiocytes which become fixed in position after wandering to the valve surface. The fixation of the cells, the presence of mobile histiocytes in the palisade, and the formation of connective tissue directly from the palisade in the valve in case 2 make this origin improbable. The most reasonable explanation is that the palisade is evolved from fibroblasts. The formation of intercellular fibrillae could be seen as the cells of the palisade grew older, and every stage toward the formation of fibrous connective tissue could be followed. Sections stained by Mallory's (aniline blue) connective tissue and phosphotungstic acid hematoxylin stains also indicate the connective tissue character of the cells.

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6. Gay, F. P., and Linton, R. W.: The Histology of Local Streptococcus Immunity. *Proc. Soc. Exper. Biol. & Med.* **23**:325, 1925-1926. Gay, F. P., and Clark, A. R.: Further Note on the Relative Protection of Polymorphonuclear and Mononuclear Cells in Local Streptococcus Infection, *ibid.* **27**:995, 1930.

The relation of the process to rheumatic infection is definite. Rheumatic lesions are said to be polyblastic and proliferative (i. e., histiocytic and fibroblastic). The lesions described here were polyblastic and proliferative. In case 2, occurring in a patient with chronic rheumatism, the process on the valve arose in association with verrucae formation in a heart that had undergone mitral stenosis following an attack of rheumatic fever, and in which young Aschoff nodules were found. In case 1, in which examination was made many years ago, before the search for Aschoff nodules became routine, there was no record of their presence or of their absence. In case 3 the lesions were inactive, but in the myocardium there were small scarred regions which might well have marked the sites of Aschoff nodules. The scarring of the valve surface in its character and location was typical of a repaired rheumatic infection. Moreover, examination of relatively acute rheumatic lesions of cardiac valves in other cases has led to the finding of at least remnants of the palisade, particularly in the interverrucal regions, in processes which had not progressed too far toward healing. The palisade seems to be an early stage of the reaction to rheumatic infection.

In case 1, in which the palisade formation was most perfect, bacteria were present on the surface of the lesion. In case 2 with a less perfect palisade no bacteria were found. In case 3 the process represented advanced degeneration of the palisade in a valve in which the adjacent layer of the surface had undergone extreme scarring, with necrosis and clotting on the necrotic area. No bacteria would be expected in such a process, and none were found.

The lesion bears little resemblance to that seen in the acute or sub-acute forms of endocarditis. The bacteria in case 1 were in smaller numbers, and their distribution was totally unlike that observed in either of these conditions. Moreover, in correspondence with the observations of Poynton and Paine<sup>7</sup> in rheumatic endocarditis, the organisms occurred on the surface as diplococci. No semblance of chain formation was discovered in the tissues. Only in culture was the formation of chains met with.

*Evolution of the Verruca.*—From the microscopic observations of the early lesions and of a series of verrucae representing various stages in the growth of vegetations from these and other cases, it is possible to follow the evolution of the vegetations from the preverrucal injury of the cell palisade to the finished product.

Injury to cell palisade manifests itself first in the form of a necrobiosis of the end-plates of the cells (fig. 3). This is followed by extension of the injury to the cell processes (fig. 4) and to the cells. Necrosis gives rise to the production of dense hyaline necrotic masses,

7. Poynton, F. J., and Paine, A.: The Etiology of Rheumatic Fever, *Lancet* 2:861, 1900.

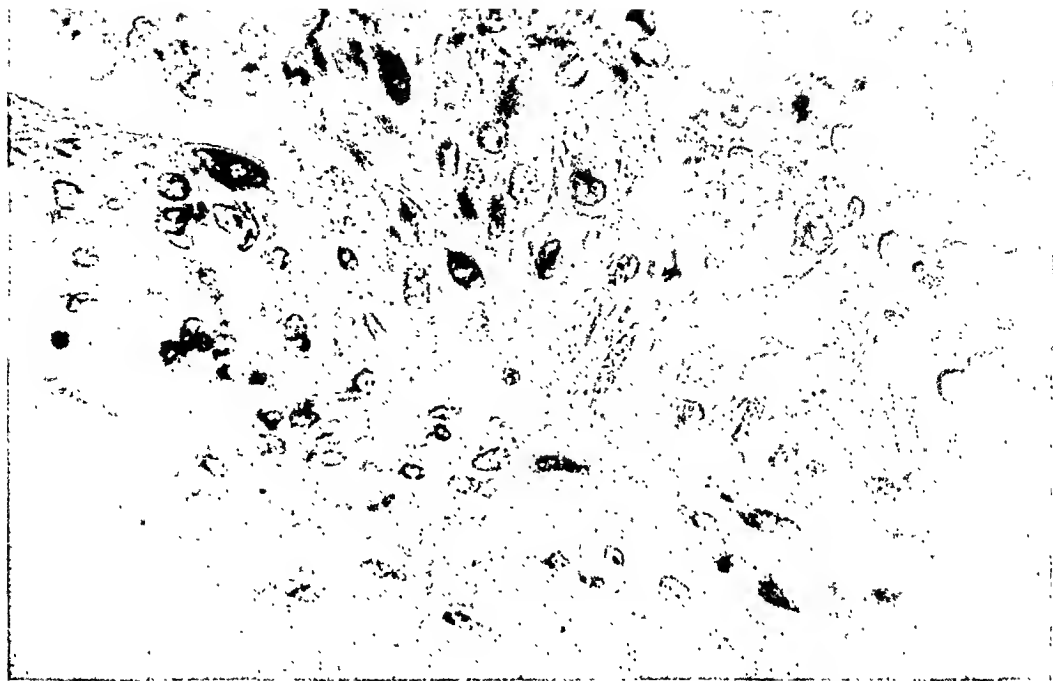


Fig. 12 (case 2).—Edge of young verruca showing fibroblastic cells;  $\times 585$ .

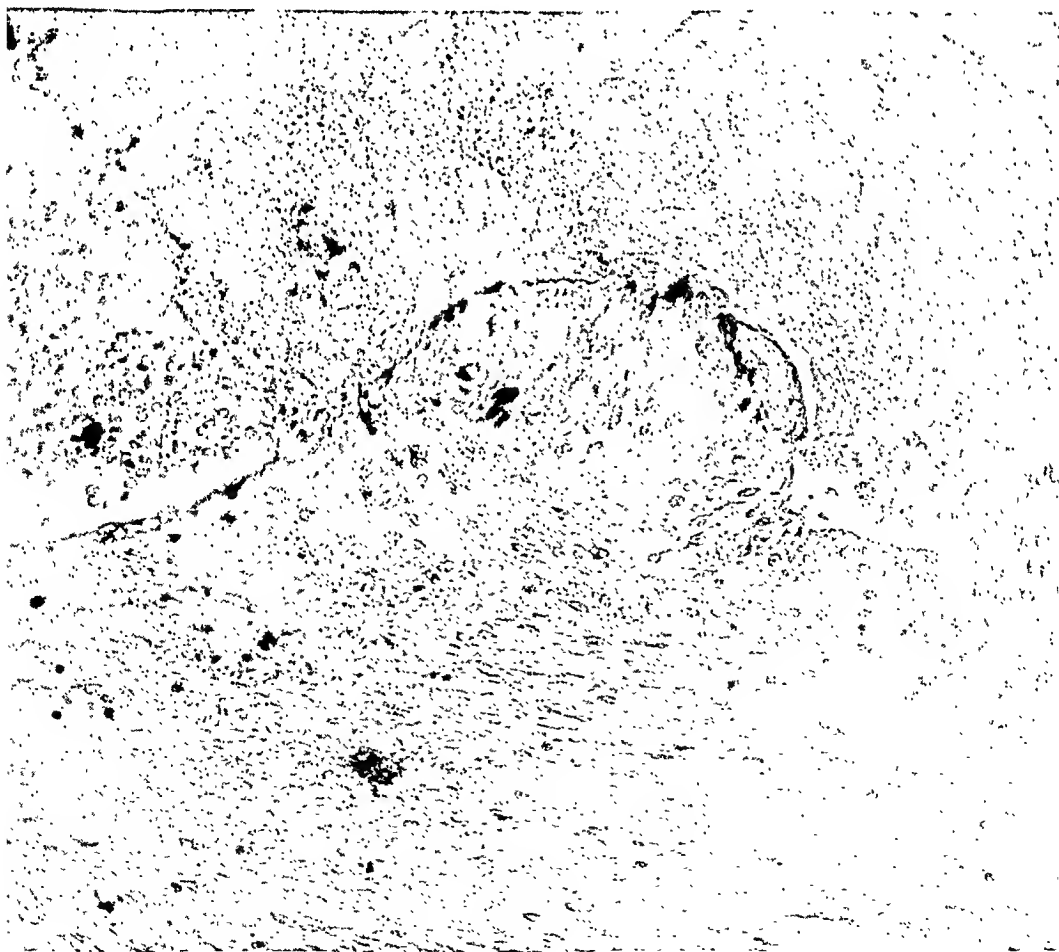


Fig. 13 (case 2).—Early stage in verruca formation; incomplete hyaline necrotic cap;  $\times 270$ .

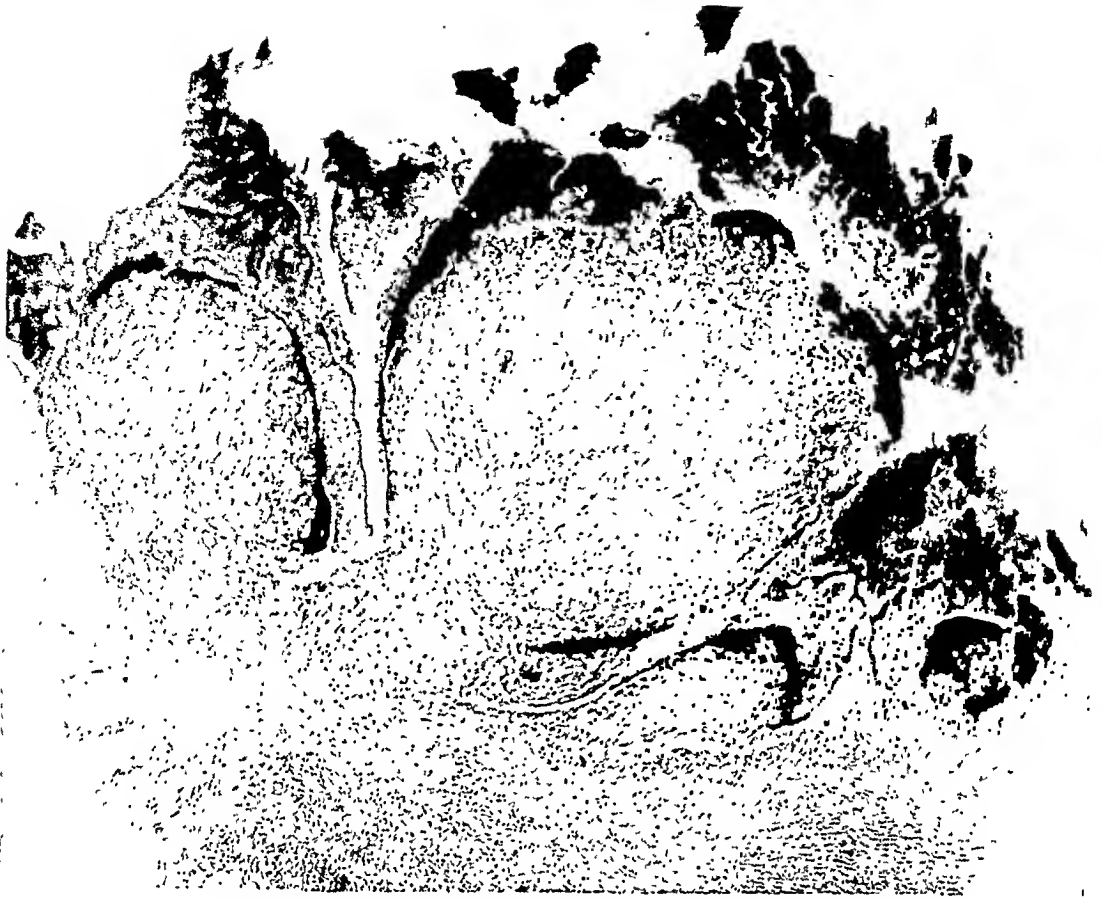


Fig. 14 (case 2).—Edge of cross-section of tricuspid valve showing four verrucae in various stages of evolution from the early budding process on the right to the relatively massive process in the center;  $\times 50$ . Note the hyaline necrotic caps.



Fig. 15 (case 4).—Verrucae on tricuspid valve in a woman, aged 26, whose mitral valve showed more advanced vegetations. Death occurred from peritonitis six days after induced abortion. There was no suspicion of cardiac disease. The vegetations ranged from pinpoint-sized growths to large ulcer-like plaques.



Fig. 16 (case 4).—Three vegetations in series in a cross-section of the valve shown in figure 15. Two almost healed verrucae and a small ulcer-like plaque are shown;  $\times 112$ .



Fig. 17 (case 6).—Mitral valve showing fused repairing vegetations—late. Compare figure 21.



Fig. 18 (case 5).—Verruca showing probable fusion of two original verrucae, in one of which almost complete organization of the hyaline necrotic cap<sup>1</sup> has taken place;  $\times 118$ .



Fig. 19 (case 2).—Old fibrous verruca showing extensive vascularization;  $\times 85$ .



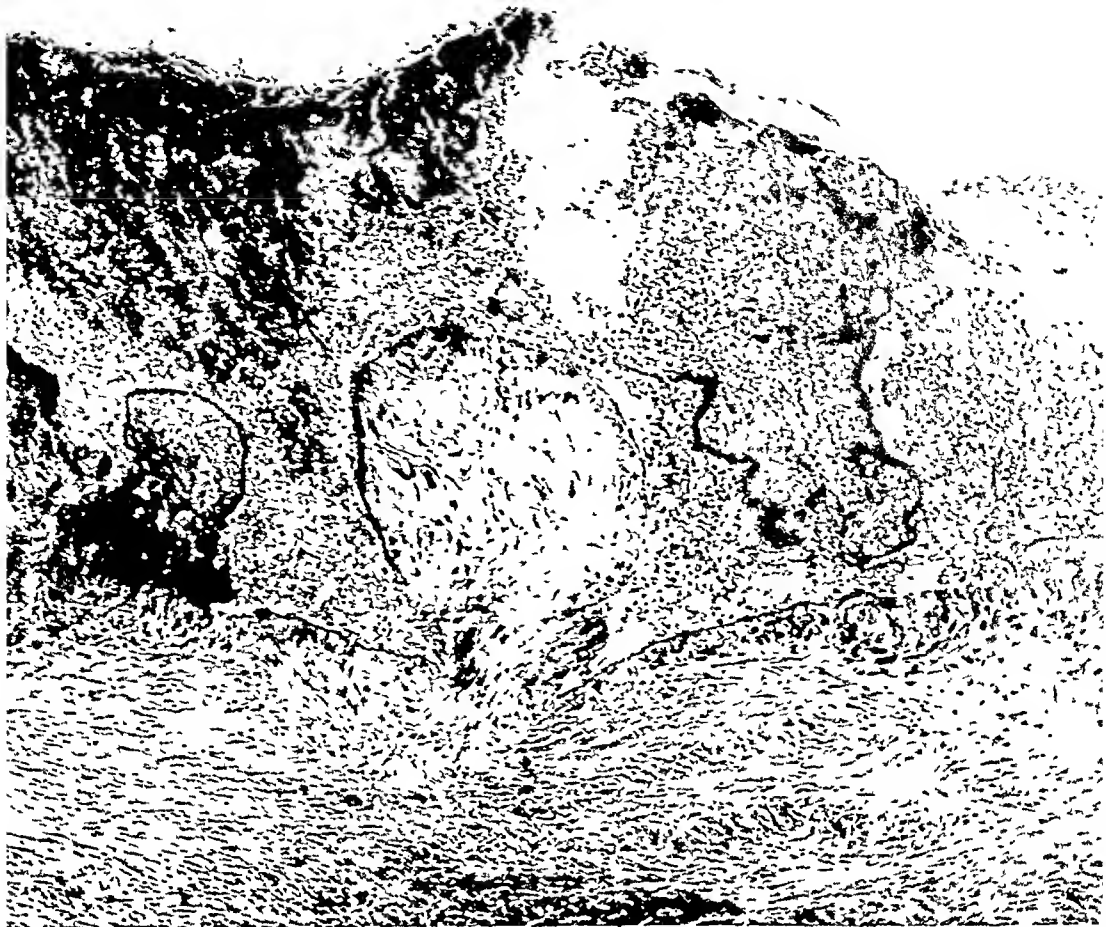


Fig. 20 (case 2).—Almost completely healed, slightly edematous (pearly) vegetation in center; early budding verruca to the left with dense hyaline cap; remains of palisade to the right;  $\times 110$ .



Fig. 21 (case 8).—Healed ridge of fused verrucae, which has not yet been drawn back into alinement with the body of the valve. Compare figure 17.



in part from cells, in part from fibrin. These masses apparently stimulate the underlying fibroblastic tissue to grow. The process is sharply focal, limited to the cells underlying the necrotic material.

The beginning of the actual formation of the verruca is marked by the burgeoning up above the surface of fibroblastic cells that project at right angles to the surface (fig. 12) beneath the hyaline necrotic masses.



Fig. 22 (case 9).—Old fibrous verruca showing remains of palisade due to more recent infection, on left edge;  $\times 200$ .

As the verruca enlarges histiocytes appear among the fibroblastic cells (fig. 13). The fibroblastic cells apparently endeavor to organize the hyaline necrotic cap, but destruction of the cap is delayed, or else accretions of fibrin to the surface of the cap occur. Only in the later stages, when the verruca is large, can evidence usually be found of the breaking up of the necrotic mass by the organizing cells.

Since the process is sharply focal, a verruca arises wherever adequate focal damage to the palisade has occurred. As a result crops of verrucae appear and may be found in series in a cross-section of the edge (fig. 14). Where the injury is more diffuse (case 4) ulcer-like plaques may occur with the characteristic growth of fibroblastic cells that project at right angles to the surface (figs. 15 and 16). As the verrucae enlarge and healing progresses, they tend to fuse and may form a continuous ridge along the contact edge (fig. 17, case 5, and fig. 18, case 6).



Fig. 23 (case 10).—Mitral valve showing diffuse scarring of auricular surface in addition to old verruca. Compare the texture of the scarred auricular surface with the relatively normal ventricular surface;  $\times 60$ .

Old verrucae may show extensive vascularization of their centers (fig. 19) or become converted into dense, relatively acellular masses of collagen. Healing is completed by the growth of endothelium over the surface. If this is realized early, there may occur edema of the fibroblastic mass, and the verruca takes on a pearly luster (fig. 20).

When healing of fused vegetations, forming a continuous ridge along the contact edge, is completed, the ridge may persist for some

time before the contraction of the connective tissue draws it back into alinement with the surface of the valve (fig. 21, case 7).

That the typical verrucose endocarditis is dependent on repeated infection is evident from the fact that vegetations showing many stages of development may be found in the same valve (figs. 14 and 20). Furthermore, old verrucae may be found with remnants of a more recent palisade on portions of their surface (fig. 22, case 8).

In addition to frank verrucae formation the cell palisade may give rise directly to the diffuse formation of scar tissue (fig. 9). Healing of this lesion may occasion wide scarring of the valve independent of the verrucae (fig. 23, case 9).

When reinfection occurs in old dense scarred valves the formation of the cell palisade and the evolution of verrucae are apparently brought about with difficulty. As a result, the verrucal processes are discontinuous. The contact edge of the anterior flap, however, is the commonest and almost a constant location of verruca formation in these valves, under reinfection, with scattered lesions on the posterior flap.

It is manifest from what has been said that the formation of the cell palisade and the production of verrucae result from injury to the surface of the valve and not from injury arising within the valve. The palisade formation is a diffuse local reaction, and its production is a response to diffuse injury of the surface such as would result from the distribution of bacteria along the contact edge as illustrated in case 1. The verruca formation is a focal response to injury at the point where the vegetation arises. Its intimate relation to the hyaline necrotic masses that are formed from locally damaged palisade cells and fibrin is apparent. Swift<sup>8</sup> indicated that the Aschoff nodule is a reaction to necrotic collagen, and there is reason to believe that the verruca has a similar provenance.

#### SUMMARY

In this paper are reported early lesions of rheumatic endocarditis. The characteristic lesion consists in the formation of a defensive palisade of cells on the contact edges of the valves. Three phases of this reaction are illustrated: one before verruca formation with bacteria on the surface of the palisade; one showing verrucae without demonstrable organisms; one with mucoid (?) degeneration of the palisade in an old, scarred valve, without organisms, and showing pseudo-verruca formation due to bland thrombosis.

The evolution of the verrucae from local regions of damage of the defensive palisade is described.

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8. Swift, H. F.: Rheumatic Fever, Hektoen Lecture, J. A. M. A. **92**:2071, 1929.

# THE FUNCTIONS OF THE GLIA IN SECONDARY DEGENERATION OF THE SPINAL CORD

THE OLIGODENDROGLIA AS PHAGOCYTES \*

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Modern trends in the investigation of glial reactions to neuropathologic processes are, in the main, toward the discovery of the function of the oligodendroglia. This aim, heretofore rather destitute of attainment, was a great factor in the determination of the present study of glial reactions in secondary degeneration of the spinal cord by the modern methods of the Spanish school.

The important phases of secondary degeneration of the spinal cord, i. e., the microchemical aspects, and the fact that the entire phagocytosis is carried out by the glial elements, were divulged by Jakob,<sup>1</sup> who earlier studied the same problem with which we have been engaged, using the more specific methods of glial impregnation. Likewise, the more characteristic phases of glial function, as related to the astrocytes and the microglia, have been presented in innumerable, now standard works by Cajal and del Rio Hortega and their school, as well as by many other authors using their methods.

More recently, del Rio Hortega<sup>2</sup> has given an encompassing insight into the structural relationships and some of the pathologic changes relating to the oligodendroglia. But aside from his anatomic studies and the researches of Penfield<sup>3</sup> and others in the purely pathologic field,

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1. Jakob, A., in Nissl and Alzheimer: *Histologische und histopathologische Arbeiten*, Jena, Gustav Fischer, 1914, vol. 5, nos. 1 and 2.

2. del Rio Hortega, P.: *Mem. r. Soc. españ. de hist. nat.* **14**:1, 1928; *Rev. neurol.* **1**:956, 1930.

3. Penfield, Wilder, in Cowdry, E. V.: *Special Cytology*, New York, Paul Hoeber, 1928, vol. 2, p. 1032; *Brain* **47**:430, 1924. Penfield, Wilder; and Cone, William: *J. f. Psychol. u. Neurol.* **34**:204, 1926.

few investigators have touched on the extremely interesting functional activity of these ubiquitous small glial cells, which are so multitudinous, and the relationships of which to the nervous tissue itself are so intimate, throughout the central nervous system.

One of the great deterrent factors in making a proper study of the oligodendroglia particularly lies in their tremendous susceptibility to the influence of pathologic processes, becoming quickly abnormal, and, in the minds of some investigators, disappearing, in the face of some pathologic disturbances of the central nervous system. Our choice, therefore, of secondary degeneration of the fibers of the spinal cord as a means of studying the functions of these and the other glial elements was furthermore guided by the fact that in this progressive pathologic process we had a means of halting the progress of the pathologic changes at definitely controllable stages for the purpose of histologic examination, and by the fact that we could avoid the postmortem complications that affect particularly the oligodendroglia in nonexperimental disease processes.

#### REVIEW OF THE LITERATURE ON THE RÔLE OF THE OLIGODENDROGLIA AS PHAGOCYTES

Present ideas regarding the normal function of the oligodendroglia cells are pretty well established. In 1921, del Rio Hortega<sup>4</sup> separated from the third element of Cajal a type of cells which he stained by a specific silver method and which he called oligodendroglia. These cells, as he showed, have few and small branches as compared with astroglia. He pointed out, moreover, that they contain a centrosome and a Golgi apparatus, possess gliosomes, and have no glia fibrils. Their processes terminate freely and without a sucker foot formation as seen in the astrocytes. They are of three main types: the interfascicular oligodendroglia, found in close contact with and among the nerve fibers; the perineuronal satellites, found around the ganglion cells of the cortex, and the vascular satellites. He recognized that these cells had much to do with the deposit of myelin in the nerve sheaths, for, as Penfield pointed out, they first appear in large numbers at the time of myelination; they are never seen before myelination begins, and at this period the protoplasmic granules are unusually large and numerous. In addition to this function, Hortega recently suggested that the oligodendroglia play a passive function of protection and isolation, separating the nerve fibers.

Hortega's work was confirmed by Penfield,<sup>3</sup> who, in addition, pointed out clearly the transitional tendencies between oligodendroglia and astrocytes, producing cells that have the characteristics of both.

In 1928, Hortega<sup>2</sup> published a comprehensive monograph in which he made a careful study of the morphologic characteristics of these cells.

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4. del Rio Hortega, P.: *Bol. r. Soc. españ. de hist. nat.* **21**:63, 1921.

These are now well known: the small, round, chromatin-rich nuclei, the concentration of the cytoplasm often at one pole, the fine processes branching dichotomously, with gliosomes at these branchings, and the close association of the processes with the myelin sheath. The last point was clearly demonstrated by Hortega, who showed that the processes follow along the myeline sheath and coil themselves around it. He believed that the oligodendroglia are of ectodermal origin, and that they take no part in the formation of the astrocytes of the nervous system. He recently divided the oligodendroglia into four classes: (1) the oligodendroglia of Robertson, from 15 to 20 microns in diameter, found chiefly around the nerve cells, but also among the fibers; (2) the oligodendroglia of Cajal, from 20 to 40 microns in diameter, not found in the gray matter, but abundant in the white substance; (3) the oligodendroglia of Paladino, which are large and are found at the base of the brain chiefly, where the largest nerve fibers are seen, and (4) certain oligodendroglia that are even larger than Paladino's, and that are also found in the cerebral peduncles, pons and medulla.

Hortega<sup>5</sup> (1922 and 1928) suggested that the oligodendroglia are similar in function to the cells of the sheath of Schwann.

The oligodendroglia were found in the optic tracts by Lopez.<sup>6</sup>

It is the function of the oligodendroglia in pathologic conditions that is still a matter of great controversy. One of the most debated problems is whether the oligodendroglia can assume the rôle of phagocytes under certain disease conditions. There are two schools: the one supported by Hortega,<sup>2</sup> Penfield,<sup>3</sup> Creutzfeldt and Metz,<sup>7</sup> Alberca<sup>8</sup> and de Asúa<sup>9</sup> maintains that the oligodendroglia cannot assume the rôle of phagocytes; the other, composed of Jakob,<sup>1</sup> Schaffer,<sup>10</sup> Ferraro and Davidoff,<sup>11</sup> von Meduna<sup>12</sup> and Pruijs,<sup>13</sup> holds that the oligodendroglia can play the rôle of so-called "gitter" cells, or "fettkoernchen" cells, i. e., phagocytes of the nervous tissue and of the fatty products of its degeneration. Jakob, indeed, described a similar activity on the part of the astrocytes; more recently, Winkler-Junius, quoted by del Rio Hortega, took the same stand.

5. del Rio Hortega, P.: *Bol. Soc. españ. de biol.*, 1922, vol. 10, pt. 1.

6. Lopez, E. M.: *Bol. r. Soc. españ. de hist. nat.*, 1926, vol. 26, no. 5.

7. Creutzfeldt, H. G., and Metz, A.: *Zentralbl. f. d. ges. Neurol. u. Psychiat.* **38**:416, 1924; *Ztschr. f. d. ges. Neurol. u. Psychiat.* **106**:18, 1926.

8. Alberca, R.: *Bol. Soc. españ. de biol.* **11**:81, 1926.

9. de Asúa, Jimenez: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **109**:354, 1927.

10. Schaffer, K.: *Ztschr. f. Anat. u. Entwcklngs.* **81**:715, 1926.

11. Ferraro, A., and Davidoff, L. M.: *Arch. Path.* **6**:1020, 1928.

12. von Meduna, L.: *Arch. f. Psychiat.* **82**:123, 1927.

13. Pruijs, W. M.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **108**:298, 1927.

Hortega believed that the oligodendroglia serve as a means of sustenance around the medullary tubes, and that their protoplasm elaborates a specific product which influences the trophic function of the nerve fibers and the elaboration of myelin. Injury to the axon and its surrounding sheath can result only in the death of these symbiotic cells. They are powerless to assume a phagocytic function. This function is possible only on the part of the microglia. His point of view has been supported by Penfield,<sup>3</sup> who denies phagocytic power to the oligodendroglia. Creutzfeldt and Metz<sup>7</sup> were unable to establish the formation of phagocytic cells from the oligodendroglia, and Struwe<sup>14</sup> found that while all neuroglia can harbor lipoids, only the microglia can form phagocytes.

Recently, Ferraro and Davidoff<sup>11</sup> were able to trace the formation of phagocytes from oligodendroglia in injuries to the brain. The determinant of this process consists in the severity of the lesion. Milder injuries result only in acute swelling of the oligodendroglial cell. It is only when the myelin sheaths have been destroyed that the oligodendroglia assume a fat-digesting function and become compound granular corpuscles. Ferraro and Davidoff were able to follow these developments by proper impregnations. Less extensive works indicating a similar function on the part of the oligodendroglia have been carried out by Schaffer,<sup>10</sup> who based his conclusions on the activity of the oligodendroglia in pathologic conditions. Von Meduna<sup>12</sup> took a similar stand and even went so far as to state that the oligodendroglia have the ability to phagocytose degenerated microglia. Globus,<sup>15</sup> on the other hand, was unable to find evidence of phagocytic properties on the part of the oligodendroglia, at least in chronic vascular disease. This rôle was played entirely by microglia. More recently, Roussy, Lhermitte and Oberling<sup>16</sup> concluded, on the basis of studies of cerebral softening, that the phagocytes of the brain are derived chiefly from the microglia, but that the oligodendroglia and the astrocytes also form phagocytes, though to a much less extent than the microglia. Similar conclusions have been reached by Roussy, Oberling and Raileanu.<sup>17</sup>

Jakob in his work on secondary degeneration did not refer to the various glial types by their present names, but by reference to the cells that are now called astrocytes, microglia and oligodendroglia, respectively, as the "protoplasma richest," "protoplasma poorer" and "protoplasma poorest," he made his findings rather clearly translatable into the modern terminology. In Jakob's comprehensive study of the afore-

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14. Struwe, F.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **100**:450, 1926.

15. Globus, J.: *Arch. Neurol. & Psychiat.* **20**:14, 1928.

16. Roussy, G.; Lhermitte, J., and Oberling, C.: *Rev. neurol.* **1**:878, 1930.

17. Roussy, Oberling and Raileanu, quoted by Roussy, Lhermitte and Oberling (footnote 16).

mentioned process in the spinal cord, made with the older, purely protoplasmic stains, he also used the term "myeloclast" to designate those cell groupings the duty of which it is to break down the axomyeline structure that has lost its functional capacity as the result of the severance of its continuity. To the secondary scavenger of this preliminarily worked-over nerve detritus he applied the term "myelophage." To the cells thus active in the third stage of the digestion and removal of this material he applied the term "'fettkoernchen' cells alpha." To the "protoplasma richest" cells that, in the later stages of the degeneration, absorb the fatty end-products of the foregoing phagocytic activities of the smaller cells he applied the term of "'fettkoernchen' cells beta." To these fat-filled cells in the still later stage of this same process in which they deliver their burden to the vascular system he gave the name of "'Fettkoernchen' cells gamma." It is this special terminology that we shall use in modified form in describing the cells and cell complexes that we have been able to differentiate more clearly, and to name with the modern terms, by means of the specific metallic impregnation methods.

#### METHOD

Rabbits were used in our investigation because of the relative ease of impregnation of the nervous system of these animals, and because Jakob's original work on secondary degeneration of the spinal cord was carried out on these animals. Hemisections of the cord were performed under aseptic conditions. The animals were killed so that the glial reactions could be studied in daily stages from the third to the eighth day inclusively, and on the tenth, twelfth, twenty-fourth, thirty-fifth, fifty-second and seventy-eighth days after hemisection. Our histologic studies were carried out entirely by the metallic impregnation methods of the Spanish school, with the Jakob-Mallory method as a control.

In order to obtain the best possible impregnations of the oligodendroglia, we used not only the original method of Hortega, but also that of Penfield and of Kanzler. Fixation in a formaldehyde bromide chloral hydrate solution <sup>17a</sup> devised by one of us (Dr. Cramer) gave good results when other methods of fixation failed. Fat stains were made at most stages of the process, usually after the silver or gold impregnations. Cajal's gold chloride mercuric chloride method was used at all stages of the investigation. The method of hemisection was chosen in order that we might have a comparison of normal and abnormal reactions in each half of the cord. For the silver methods, this was particularly useful, since the normal half of the cord always gave us suitable control material for the determination of the success of our impregnations. Without it, we should often have worked in the dark.

In our description of the processes, we shall use the terminology of Jakob referred to, and in view of the comprehensiveness of that author's work, we shall neglect for the most part to refer to the purely chemical aspects of the study, except as they deal directly with the activities of the glia.

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17a. The formula for the formaldehyde bromide chloral hydrate solution is as follows; ammonium bromide, 32 Gm.; solution of formaldehyde, 225 cc.; chloral hydrate, 20 Gm., and water to make 1,100 cc.



## REPORT OF OBSERVATIONS

EARLY STAGES: THIRD TO FIFTH DAY—FORMATION OF  
MYELOCLASTS

The first pathologic changes to be seen in the areas destined to degenerate are exhibited by the axis cylinders, which frequently impregnate with silver carbonate and gold sublimate; these changes are: swelling, distortion and coil formation. Usually, before the cylinders break up, they display an increasingly heavy-staining granular appearance. Accompanying this, as the first cellular reaction to be seen, are the changes that occur in the oligodendroglia, which will be discussed under the headings Destructive Phase and Proliferative Phase.

*Oligodendroglia.*—Destructive Phase: The primary swelling of the entire body of the oligodendrogliaocyte is an astoundingly acute and great reaction, in the face of an otherwise quiescent-appearing field. As typically illustrated in figures 1 to 4 inclusive, taken from gold sublimate and silver carbonate preparations, this early reaction causes the oligodendrogliaocytes to become as large as the astrocytes, so far as the cell bodies are concerned. Accompanying this swelling of the body is also a swelling of the nucleus, which shows a tendency to become eccentrically placed. Usually the nucleus becomes darkly impregnated, but may, less often, be more lightly impregnated with silver carbonate than the normal. Whereas the oligodendroglia may be seen in the gold sublimate preparations, lying in rows between the fully impregnated macroglia, they may be carefully studied only in the silver carbonate stain, because only there do their processes also become impregnated. These prolongations from the cell body can usually be followed only a short way, but sometimes they can be traced for tremendous distances.

In contrast to the rather finely and evenly granular appearance of the swollen cell body, the nucleus is distinguished by heavily staining granules, which are more numerous about the edges, but which tend to give rather characteristic markings to the more lightly impregnated center portion, by extending centripetally in wavy, tongue-like forms. The fine prolongations are, as a rule, difficult to trace for any great distance from the body in the ordinary silver carbonate preparation. But occasionally these prolongations become hyperargentophilic, especially on the occasions when an oligodendrogliaocyte is lying closely approximated to an axis cylinder that is in the first stages of swelling. One such example is illustrated, though only in part of its massiveness, in figure 4 *A*.

The first myeloclasts are formed from the type of oligodendroglia just described, i. e., from those lying almost in direct union with the cylinder. In figures 3 and 4, representing the fifth day of degeneration, the subsequent changes can readily be seen. The hyperargentophilia

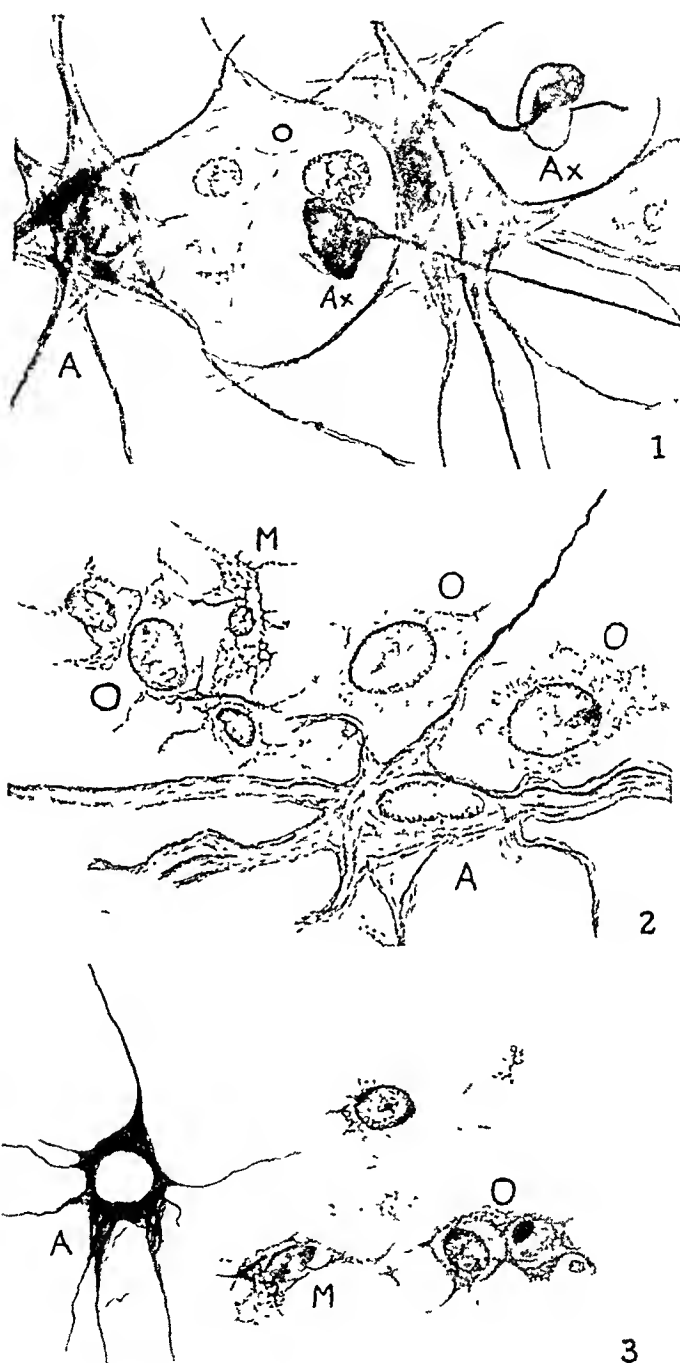


Fig. 1.—Secondary degeneration of the rabbit spinal cord at three days: In section 1 are two axis cylinders (*Ax*) in the process of degeneration in association with three oligodendroglia (*O*) in the process of acute swelling (gold sublimate method). In sections 2 and 3 are swollen oligodendroglia (*O*) and a swollen microglia cell (*M*). The astrocytes (*A*) are normal (silver carbonate method of del Rio Hortega); camera lucida drawing;  $\times 1,300$ .

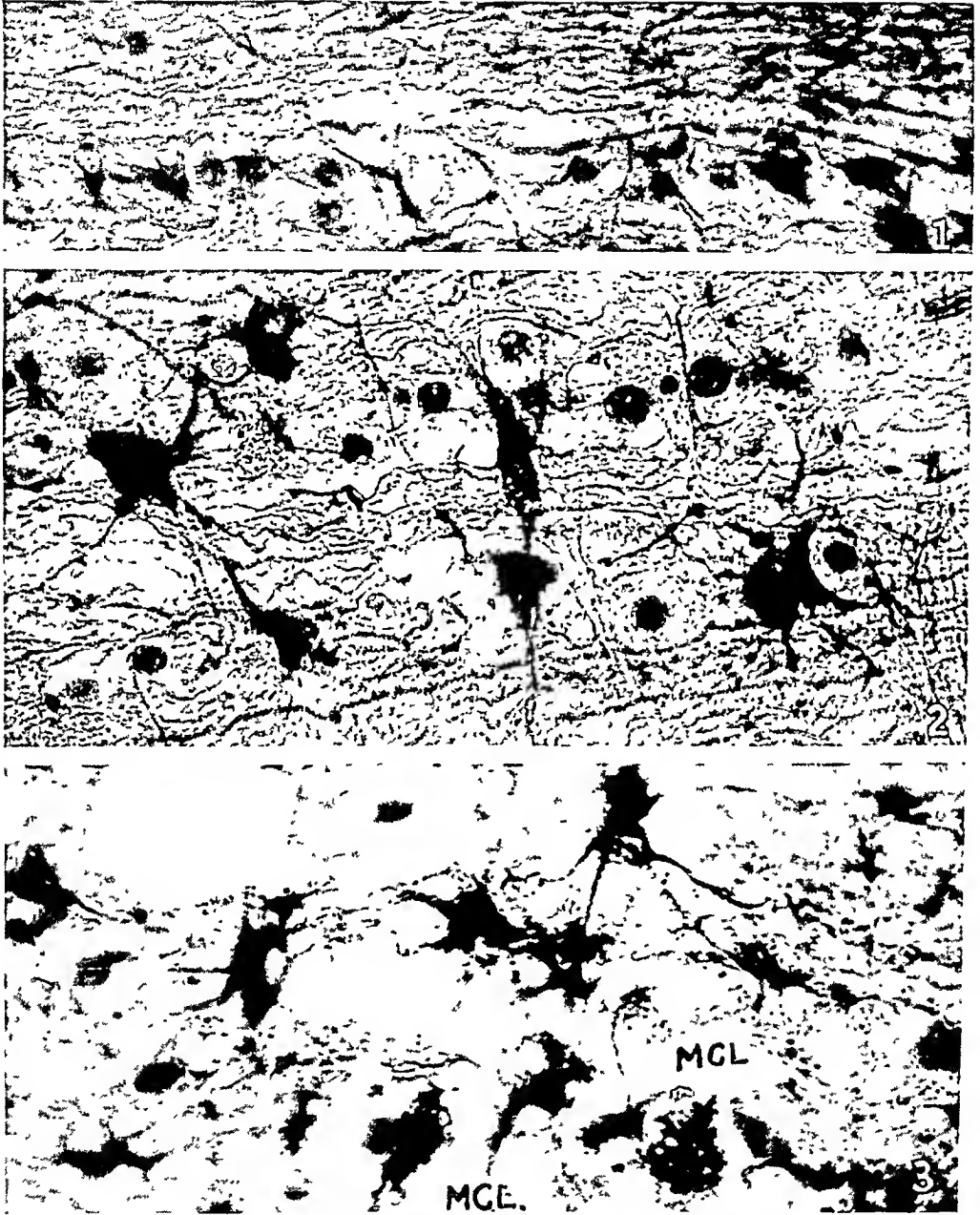


Fig. 2.—Section 1 is normal rabbit cord showing the orderly arrangement of oligodendroglia and astrocytes in the gold sublimate preparation. Section 2 represents the third day of secondary degeneration, showing large astrocytes and acutely swollen oligodendroglia still arranged in rows (gold sublimate method); section 3, the fifth day of degeneration, showing several hypertrophied astrocytes and two early myeloclasts (*MCL*), i. e., swollen, disintegrating axis cylinders with which acutely swollen oligodendroglia have amalgamated; silver carbonate method of del Rio Hortega.

of the processes is associated with a similar reaction on the part of the cytoplasm. The latter, however, in its acute swelling, tends to become quickly dispersed, and within its widened area are no longer seen the scattered dark granules, but a spokelike arrangement of granular matter, heavily impregnated with silver, and ending in the periphery (now already indistinguishable from the material of the axis cylinder itself) in a weblike manner. These intracellular "spokes," as we choose to describe them, or "girders," as they have been described by others, are to be definitely distinguished from the extracellular prolongations. The latter, arising from the cell bodies, are to be seen only in the normal, and

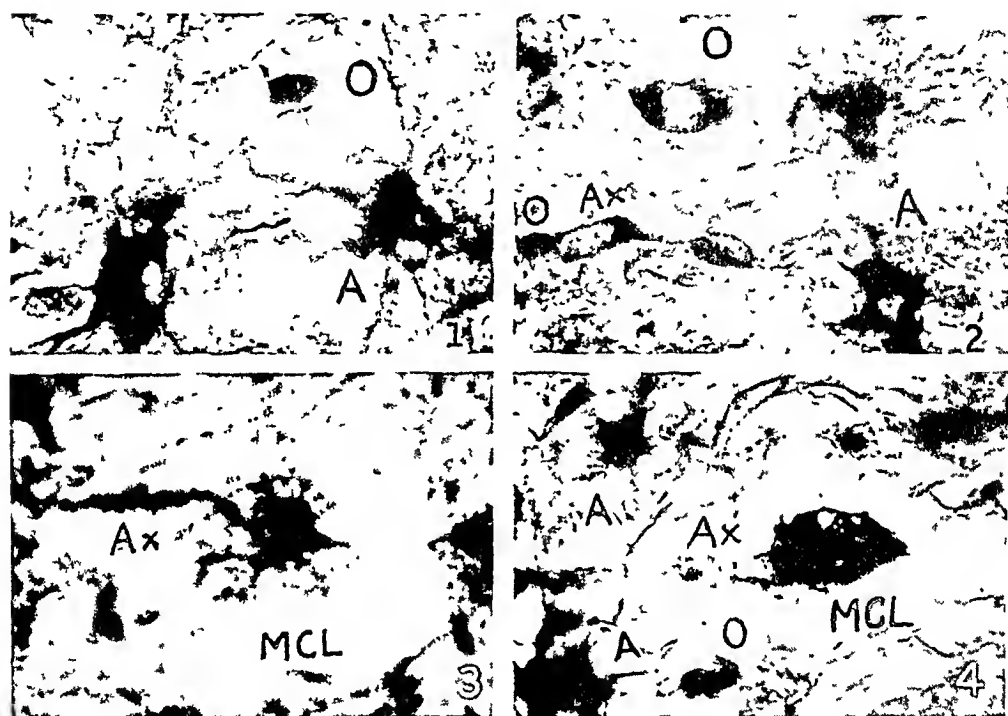


Fig. 3.—Secondary degeneration at five days: Section 1 shows normal oligodendroglia (*O*) and two hypertrophying astrocytes (*A*). Section 2 shows swollen oligodendroglia (*O*), axis cylinder (*Ax*) and astrocytes (*A*). Section 3 shows disintegrating axis cylinder (*Ax*) and swollen, wheel-like oligodendroglia—the beginning myeloclast (*MCL*). In section 4 is the completed primary myeloclast (*MCL*) from the oligodendroglia; silver carbonate method of del Rio Hortega;  $\times 400$ .

in the beginning abnormal, state of the cell; they become quickly lost with the acute swelling of the cell. The girders, on the other hand, extend from the nucleus to the limiting cell membrane and are only to be seen in swelling of the oligodendroglia. Instead of being ultimately dispersed away from the cell and from each other, as is the case with the prolongations, they tend to become confluent as they near the cell membrane, and this confluence builds the weblike pattern that is seen in these swollen cells.

The final stages of this phase of the destruction of the oligodendroglia and the building of the primary myeloclast are characterized by a karyorrhexis and cytolysis on the part of the cell and an argentophil granular degeneration on the part of the swollen neural tube, the two elements together forming a large, darkly staining, webbed ball of mixed neural and glial detritus.

These same darkly impregnating nuclei may be seen as a part of the beginning myeloclast in Cajal's gold sublimate preparations, in which the axis cylinders and their sheaths are usually impregnated, as well as the nuclei and cell bodies of the oligodendroglia, without the processes of the latter, however.

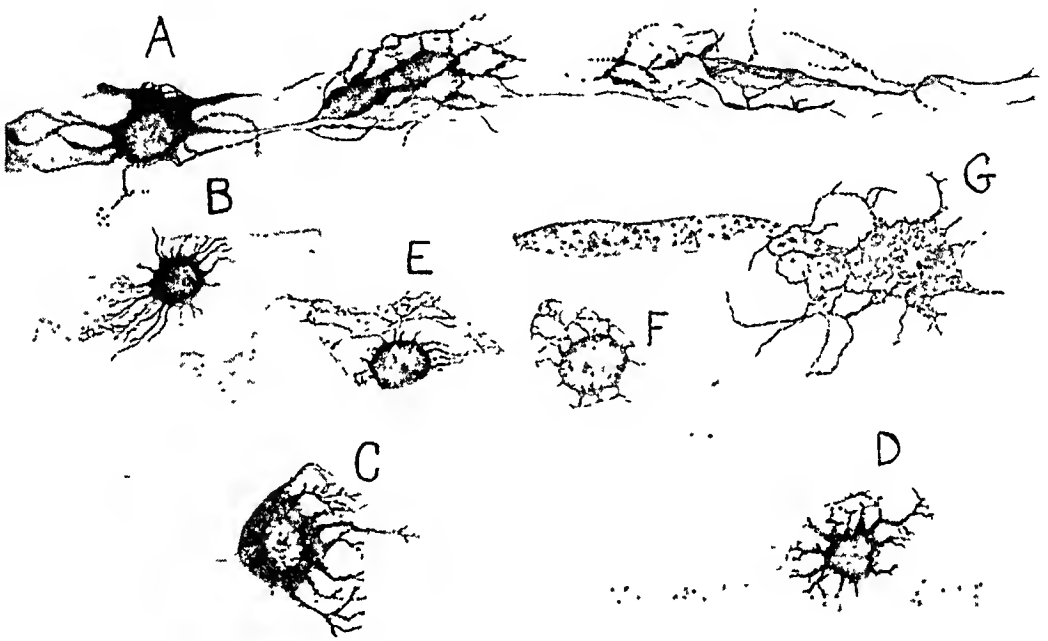


Fig. 4.—Secondary degeneration at five days, showing the various stages of formation of the primary myeloclast from the oligodendroglia and degenerating axis cylinders. *A* and *B* show oligodendroglia with hyperargentophilic processes, lying on a swollen axis cylinder. *C* shows the cell with greatly swollen processes. *D*, *E* and *F* illustrate the disintegration of the cell body and processes and their disappearance into the axomyeline structure. *G* shows the final stage in which the nucleus is also beginning to degenerate.


**Proliferative Phase:** In these earliest stages, the proliferative phase is seen as the reaction of the oligodendroglia that do not lie in direct connection with actively degenerating axis cylinders; it consists of a more moderate swelling than that described, also of a definite, although at this early stage slight, hyperplasia.

**Microglia.**—The smallness of number and the slowness of reaction of the microglia in this relatively active degenerative process has been

an astounding observation from the beginning of our studies. The fact that the microglia are strikingly few in the white matter of the spinal cord has been noted by other authors, and this relative absence of these more generally recognized scavengers (even in the presence of active pathologic processes) has been the basis for a proposal that the macroglia take over the function of the microglia in such cases. This is a proposal with which we must heartily disagree, in the case in hand. As we have shown, it is the oligodendroglia that play the initial cellular rôle. Despite the modesty of their number and the delay in their activity, the microglia are not only demonstrably present, but are also functioning as phagocytes. They are distinctly in the minority, as compared with their ectodermal neighbors, and therefore less conspicuous; but aside from these restrictions, they are individually none the less active. A question of theory as to the source of certain groups of microglia in unwonted number outside the areas of most active degeneration and seeming bent on entering these areas will be discussed later.

Once the microglia have made their entrance into the myeloclastic area, they react typically, but always more slowly than in the neuropathologic fields more frequently studied than this one. After undergoing generalized swelling and loss of the finer branches and brushes, they assume a locular, spongelike appearance and proceed to take within their bodies the particles of neural and glial detritus making up the myeloclasts. Their cyto-architecture remains distinct from that of the oligodendroglia that have undergone swelling, and the differences between the two types of cells, both of which undergo swelling and intracellular changes to the point where they may be spoken of as "gitter" cells, can be seen distinctly almost to the point of cytolysis. Whereas the sensitive and quickly reacting oligodendrogliaocytes are more or less globular from the beginning and have their typical spoke-like cytoplasmic structures about the round nucleus, the microglia retain their elongated form for a long time. The arrangement of the lacunae within the gitterizing microglia is irregular, resembling that of the lacunae of a sponge. The oligodendroglia, on the other hand, have the more weblike appearance described.

*Macroglia.*—In contrast to the oligodendroglia and the microglia, the macroglia display, from the beginning of the secondary degeneration, an aspect of sturdiness in their reaction. But they, too, react to the stimuli that arise from the disintegration of the combined neural and glial material of the myeloclast by becoming hypertrophied and hyperplastic. Both cell body and nucleus become enlarged, and the latter is frequently seen to divide amitotically, so that "twin" and "triplet" forms are frequently occurring phenomena in the early stages (figs. 1 [section 1], 2, 3 and 5 [sections 1 and 2]). In these early days of the fall of the



neural tubes, there is seldom any sign of clasmatodendrosis on the part of the astrocytes, a hardness that contrasts strikingly with the comparative frailty and quick fall of the other two types of glia. There is a minimal motile reaction on the part of the prolongations that surround the myeloclasts, but for the most part this is a passive mobility at first, merely in conformance to the shape and size of the swollen oligodendrogliaocytes and the axomyeline tubes.

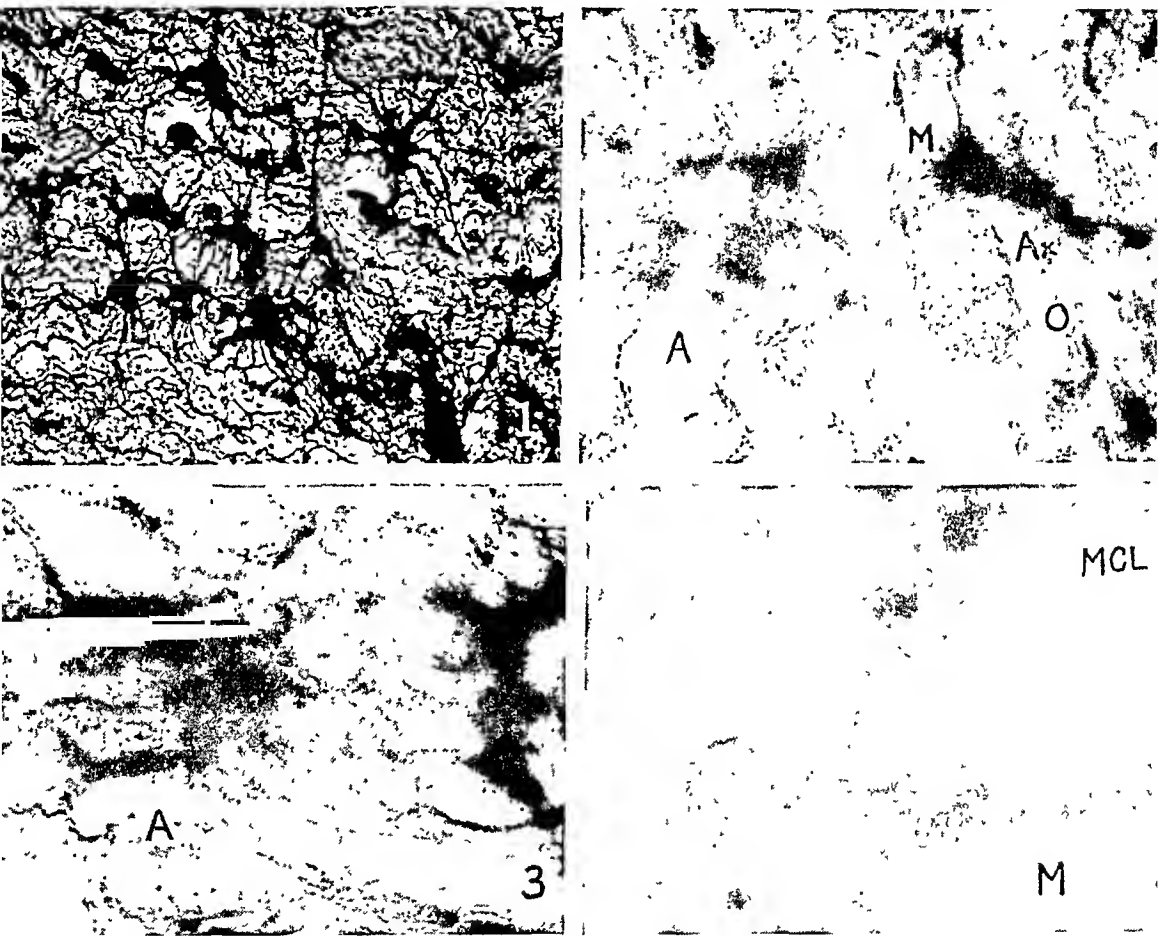


Fig. 5.—Secondary degeneration at five days: Section 1 shows hypertrophied astrocytes with some primary myeloclasts. In section 2 are seen twinning astrocytes (*A*). Sections 3 and 4 show a primary myeloclast (*MCL*) and a secondary invasion of microglia (*M*); silver carbonate method of del Rio Hortega.

#### CONTINUATION OF EARLY STAGES: FIFTH TO TENTH DAY

After the formation of the primary myeloclasts described, occurring in the first five days of the process and characterized chiefly by a degeneration of the axis cylinders, by an acute swelling of the oligodendroglia attached directly to the neural tubes and finally by the complete union of the nervous and glial elements, there is a repetition of the

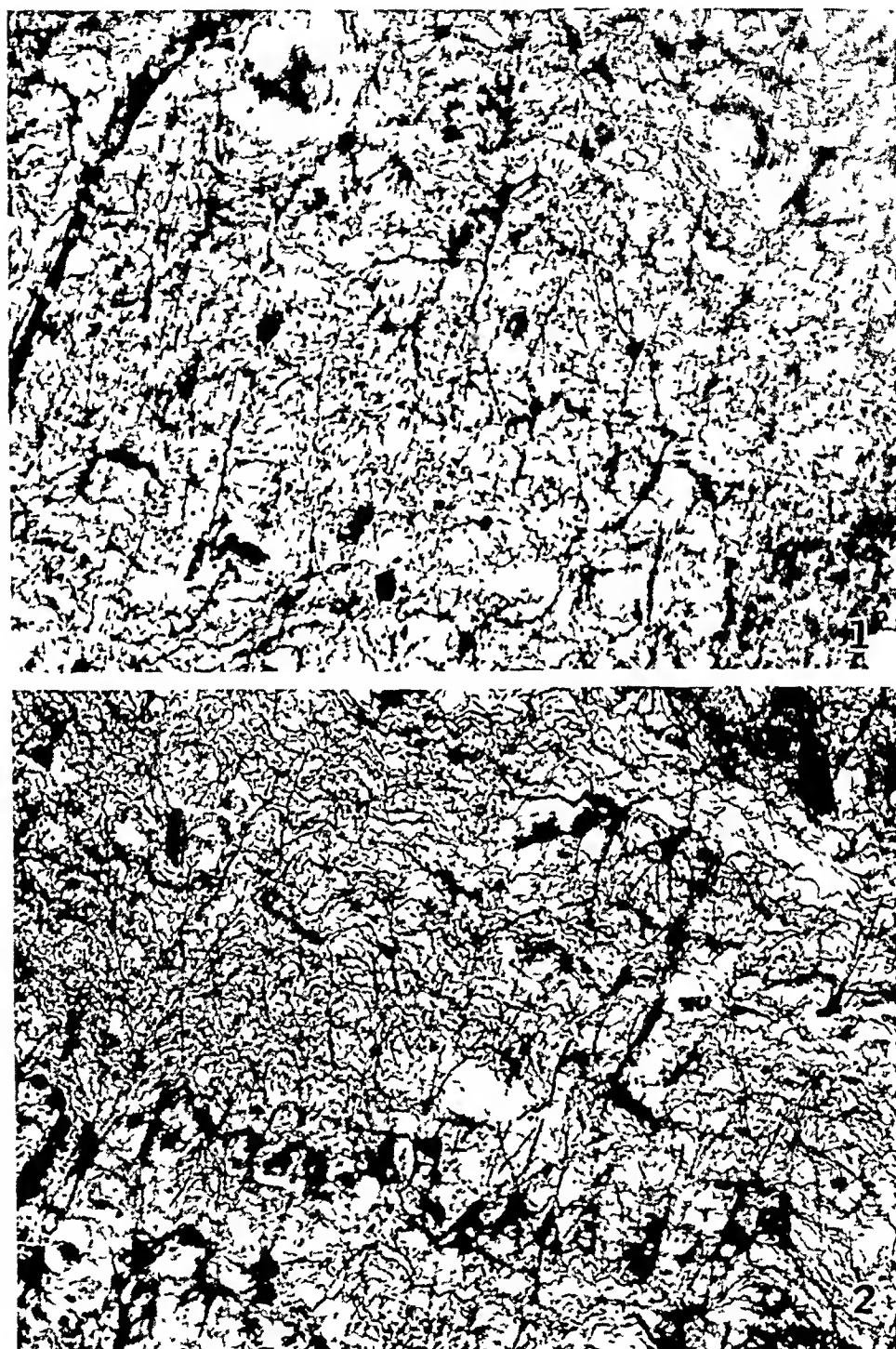


Fig 6.—Secondary degeneration at five days: In section 1, outside the area of most active degeneration, microglia are abundant; in section 2, in the area of most active degeneration, microglia are few, but oligodendroglia are forming primary myeloclasts, and astrocytes are abundant; silver carbonate method of del Rio Hortega.



same process by other oligodendroglia and by microglia. These secondary scavengers we shall speak of as secondary myeloclasts, and we consider their function to be the removal by digestion of the primary myeloclastic masses. Although these newly activated cells are the most prominent workers in the second five days, they are not the only ones that we see; for on the outskirts of the advanced areas of degeneration the signs of the reactions of the earlier stages may still be seen. This, indeed, is typical throughout the various stages examined; i. e., the early and late processes may be seen going on adjacent to each other.

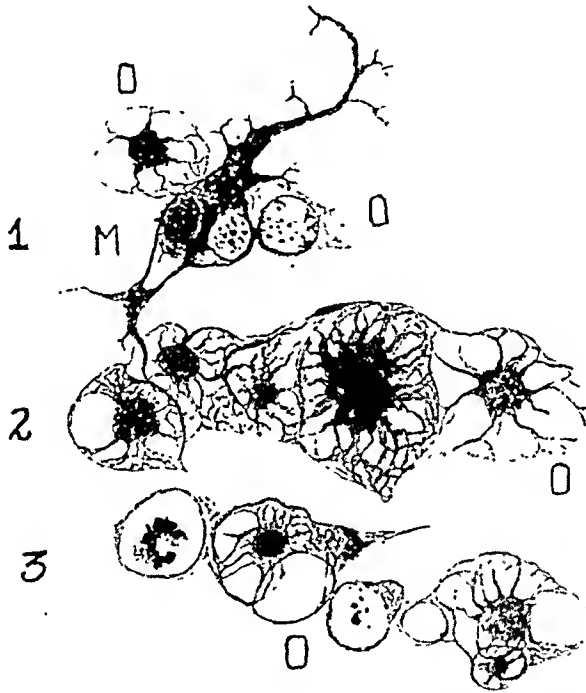


Fig. 7.—Secondary degeneration at eight days. Formation of secondary myeloclasts from oligodendroglia (*O*) and microglia (*M*); 1 and 2 are one complex; 3 is further advanced and is composed only of oligodendroglia. The differences between the two types of glitter-like cells is clearly shown.

In our descriptions of the various periods, however, we shall give only the observations salient to the most advanced reactions.

*Oligodendroglia.*—In the period from the fifth to the tenth day, the primary myeloclasts have all been formed, in the most representative areas, and surrounding them are the cells that are preparing to assume the functions of secondary myeloclasts. There is, at this time, a noticeable increase in the number of microglia present at times, as shown in figure 6, section 1, but the oligodendroglia are still in the majority.

The oligodendroglia that are active as secondary myeloclasts are similar to those that were last described as, in the period before the

fifth day, lying in rows and groups and undergoing hypertrophic changes. Here they react further by the loss of prolongations and the formation of spokelike and weblike intracellular structures. Lying close together in rows, they tend to coalesce, and expanding as coalesced groups, they overtake the degenerated materials of the former axis cylinders, myelin sheaths and oligodendroglial and microglial rests of the preceding period. Three such groups are illustrated in figure 7, taken from a preparation of the spinal cord at the eighth day of degeneration. It shows not only the distinctly contrasting form of the microglial cell that is present, but also the temporality of the oligodendroglia, going quickly to ground while still performing their duties. This is demonstrated by karyorrhexis in the case of two of the cells in the more advanced group.

*Microglia.*—The microglia that are present at the site of the primary myeloclast or those that, migrating from the adjacent tissue, enter the myeloclastic process secondarily are, in the end, as ill-fated as the oligodendroglia; their swollen, degenerating bodies and nuclei remain in the myeloclastic process. They are replaced, however, by fresh microglia that enter the old process to add more phagocytic bulk to the incompletely fermented, degenerating myeloid material. One interesting arrangement of these new microglial cells as a whole is that of a ring-like disposition about the myeloclast as shown in figure 8. Either one or two microglia are frequently seen to encircle these structures, and though they do so in company with macroglia, the prolongments of which bend about myeloclasts also, as though to wall them off from the adjacent intact neural tubes, the microglia are sometimes the only encircling structures to be discerned in the silver carbonate stain. When the microglia are seen in this encircling form, they are, except for their unusual shape and the absence of many of the fine branches, still relatively normal in structure. They gradually disappear, however, into the mass of the myeloclast and, like the oligodendroglia before them, become, in time, degenerated cells.

*Macroglia.*—In the tenth and twelfth day stages of the degeneration, there is a remarkable change in the architectural aspect of the myeloclastic process. This is brought about chiefly by the astrocytes, which, in the fifth day stage, were merely undergoing hypertrophic and proliferative changes in situ; they now begin to show a greater compactness of the cell bodies of certain of their number and to display mobile tendencies in their prolongations, which are now arranged in a ring-like manner about the myeloclasts. This is illustrated graphically in figure 8, which compares two complexes of active glial cells from the cord at the eighth and twelfth days of degeneration, respectively. In the drawing from the twelfth day stage, the processes of the macroglia

can be seen to bend themselves about the myeloclast, as the leaves of a sun-sheltered plant bend toward the light. Figure 9 shows the somewhat more completed process of ring building by the astrocytes, as seen photomicrographically. It will be noted that these fibrous prolongments of the astrocytes show a rather marked fibrosity, which again emphasizes the essential sturdiness of the macroglia in this process. The macroglial radiations participate in this enclosure-building process in all directions about the cells, so that by following the radiations, by in-

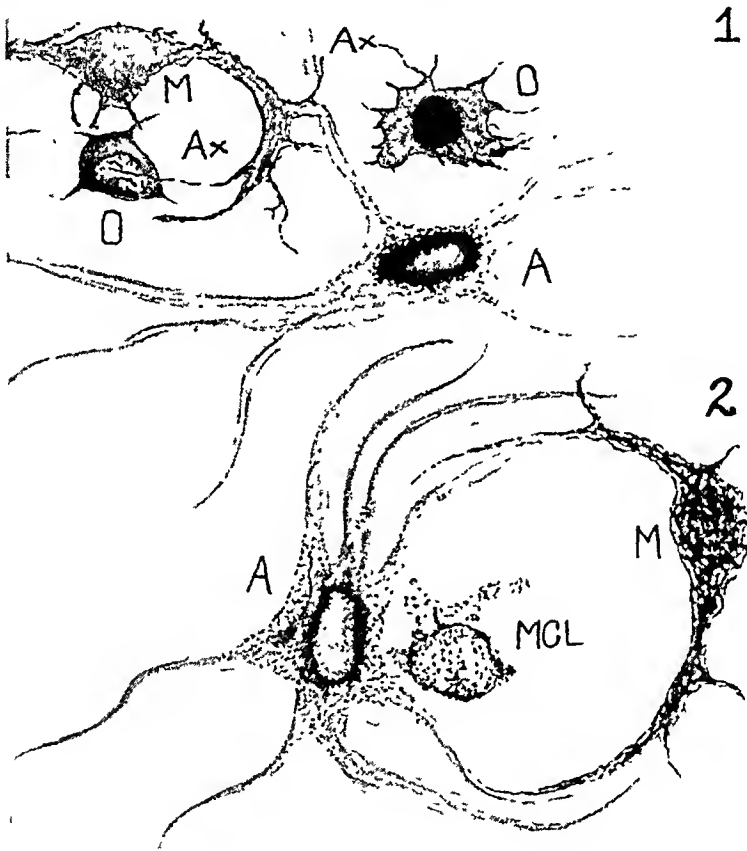


Fig. 8.—Secondary degeneration at eight days in 1, showing the various participating elements. In 2, the degeneration has gone on for twelve days and shows a hypertrophied astrocyte (*A*), a swollen microglia cell (*M*) and a myeloclast (*MCL*).

and-out-focusing, into the various planes of the cell's extent, one may see the cell nucleus and its surrounding cytoplasm as the center of a system of thin-walled globules.

Not infrequently, however, one also sees evidences of a more than structural participation of the macroglia in these areas of digestion of fat, i. e., there are occasional signs of a partial clasmatodendrosis of some of the macroglial prolongments. As seen in this stage alone,

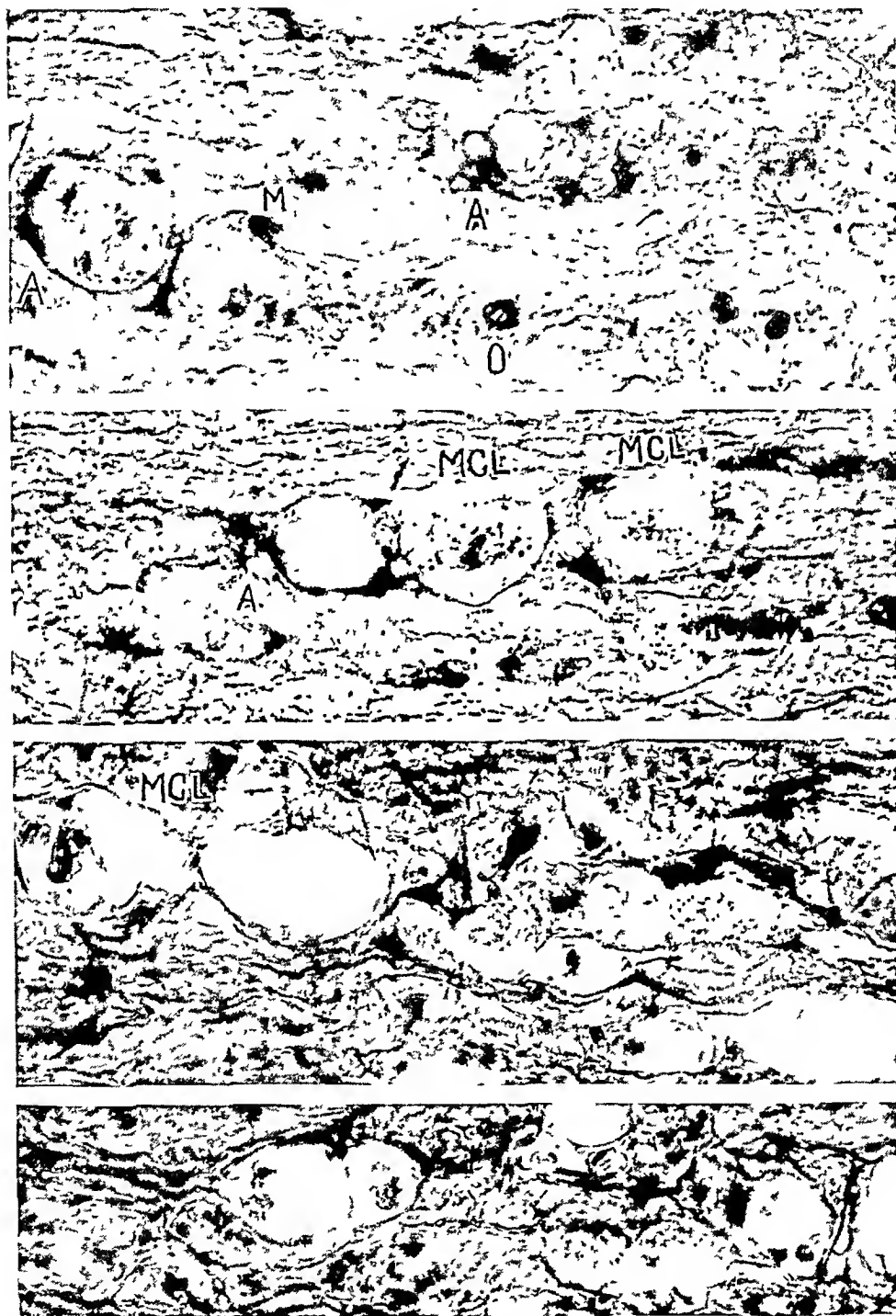


Fig. 9.—Secondary degeneration at twelve days, showing astrocytes (*A*) building rings about myeloclasts (*MCL*).

this may appear the result of pure circumstance, due to an overwhelming toxic involvement of individual prolongations, which could not withstand the chemical destructive activities within the myeloclasts; for it is seen as a phenomenon associated with certain more protoplasmic-appearing than fibrous-appearing astrocytes. These seem to be relatively newly formed astrocytes; their bodies and nuclei are more rounded than the ordinary polygonal, strongly fibrous macroglia, and though fibrous to a certain extent, they contain finer and fewer fibers than the other, older-appearing macroglia. Another and more remarkable fact about these so-called protoplasmic astrocytes, which heralds the remarkable changes to be seen in the much later stages of astrocytic function, is that vacuoles occasionally occur in their cytoplasm, as well as certain inclusions that, to use a dangerous comparison, resemble the nuclei of the oligodendroglia as they are seen in the gold sublimate impregnation.

#### TWELFTH TO THIRTY-FIFTH DAY—ACTIVITY OF MYELOPHAGES

From the twelfth day, i. e., about the second week, until the fifth week of the degeneration, there is a remarkable sameness about the appearance of the degenerating areas. One of the factors that enters into the causation of this appearance is the comparative retardation of activity on the part of the small scavenging cells, so that the myelophagic work proceeds at a relatively slow pace. But the principal factor in this similarity of appearance lies in the striking presence, in both gold sublimate and silver carbonate preparations, of a host of small round cells with rounded, deeply impregnating nuclei. They are present in such numbers that there is no question but that they represent an absolute increase in the glial population of the affected areas. The fact that these cells are most prominent in the silver carbonate preparations, which were meant to show the oligodendroglia more specifically, and in the gold sublimate preparations, in which the oligodendroglial nuclei are well impregnated, but in which the microglial element is probably poorly represented, does not, alone, suffice to prove that these hyperplastic cells are mainly oligodendroglia. This fact is, however, good presumptive evidence, and it is confirmed by a careful study of the architectonic qualities of these cells.

At about the twelfth day, the primary and secondary myeloclasts have resolved themselves into masses of detritus lying within the previously described macroglial rings. Within these enclosures there is now little of a decipherable cellular nature to be seen except for an occasional, somewhat belated, but active, cellular lytic process on the part of microglia, which have obviously come from a location more or less distant from the active degenerative site, for they are still to be seen

in such a well preserved form as demonstrated in figure 6, section 1. These microglia are to be considered as the last elements in the myeloclastic process, destined to become degenerated as a result of their phagocytic activity and then to lie with the other nuclear, cellular and axomyeline rests, along with the other degenerated phagocytes, ectodermal and mesodermal, the activities of which were described under the heading Myeloclasts.

On the outskirts of the myeloclasts are seen the oligodendroglia and microglia that are mobilizing there to appear within the degenerating process in successive waves, first as so-called myelophages, and later as fettkoernchen cells alpha, supplementing the work of their preceding prototypes, the work of which was more purely clastic.

These myelophages are, again, mostly oligodendroglia, but the microglia have also become relatively more numerous than in the normal cord and the earlier myeloclastic process.

There is little difference between the appearance or the mode of reaction of these small cells and that which was described for their predecessors in the earlier stages. The appearances of these myelophages of both oligodendroglial and microglial origin are shown in the drawings in figure 7, representing the eighth day stage, and figure 10, representing the twelfth day stage, as well as in the photomicrograph in figure 11, section 1, which was taken of the twelfth day stage and in the photomicrographs in figure 12, taken of the twenty-fourth day stage of degeneration. The same absolutely typical changes described for the secondary myeloclasts recur in these cells, with the following exceptions: that, as evidence of absorptive activity, the greatly swollen oligodendroglia, whether occurring singly or in coalesced groups, now contain vacuolated areas within their cell bodies, and that the relatively quick karyorrhexis and cytolysis that overtook the early oligodendrogliaocytes, which were active in a clastic capacity, are now no longer seen, as a rule. This is interpreted, not as an inherent difference in the cells, but as evidence that the products of myeloid degeneration which their predecessors attacked and helped to digest, or ferment, are now not so toxic to the cells themselves.

Whereas the cells described in the earlier stages as engaging in the phagocytic process have lost their identity from the point of view of the phagocytes themselves, by breaking up with the degenerating mass, this observation now becomes less frequent. It is true that the myelophages, i. e., those oligodendroglia and microglia that enter the degenerating zone from about the second to the fifth week, also degenerate in great part. But this cytolysis proceeds at so slow a rate that it is no longer striking, as was the case with the myeloclasts, of both the primary and the second orders.

FIFTH TO EIGHTH WEEK—FETTKOERNCHIEN CELLS ALPHA

Beginning with the fifth week there are evidences of the formation of fat within some of the myelophages—an evidence that the axomyelin and destroyed glial elements have finally reached the stage in their degen-

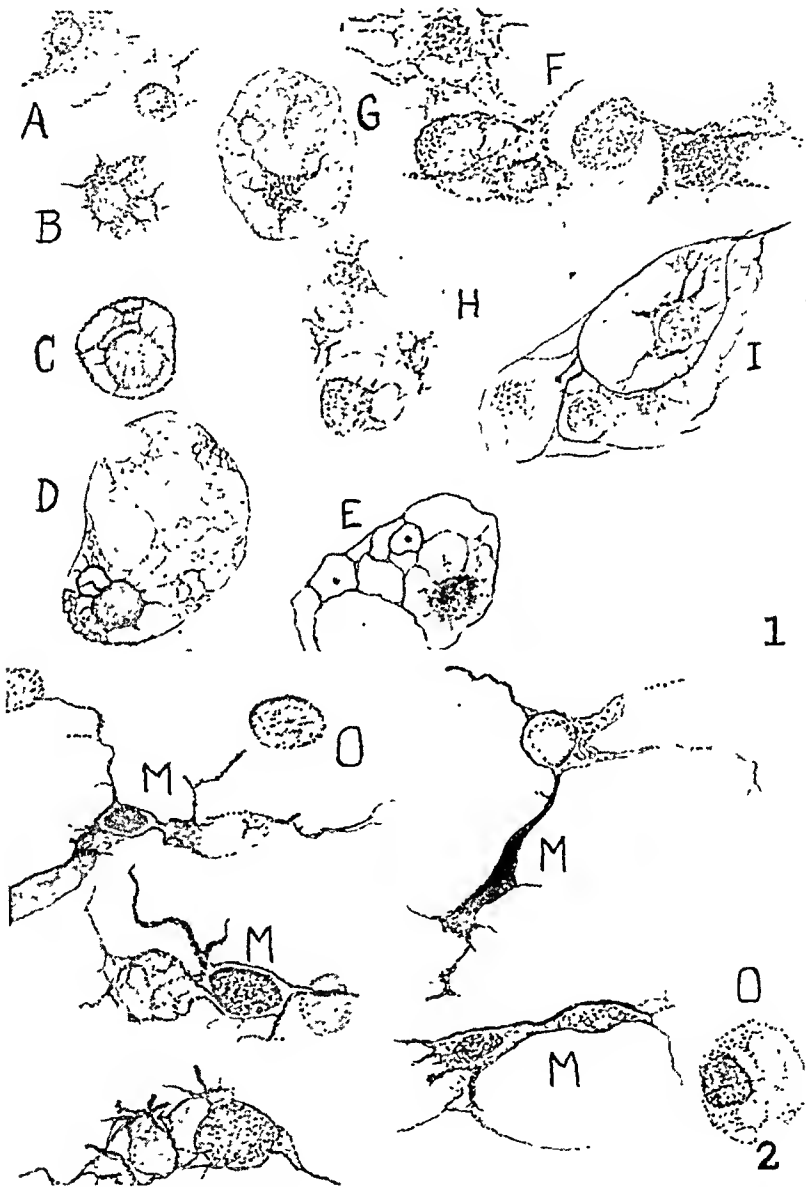


Fig. 10.—Secondary degeneration at twelve days: Section 1 shows oligodendroglia forming myelophages, singly (A-E) and in groups (F-I). Section 2 shows microglia (M) forming gitter cells.

eration in which the products of change are no longer dangerous to the existence of the cells the function of which it is to transport them away from the site of the origin and to deliver them finally to the vascular system. All three glial types will be shown to be engaged in this trans-

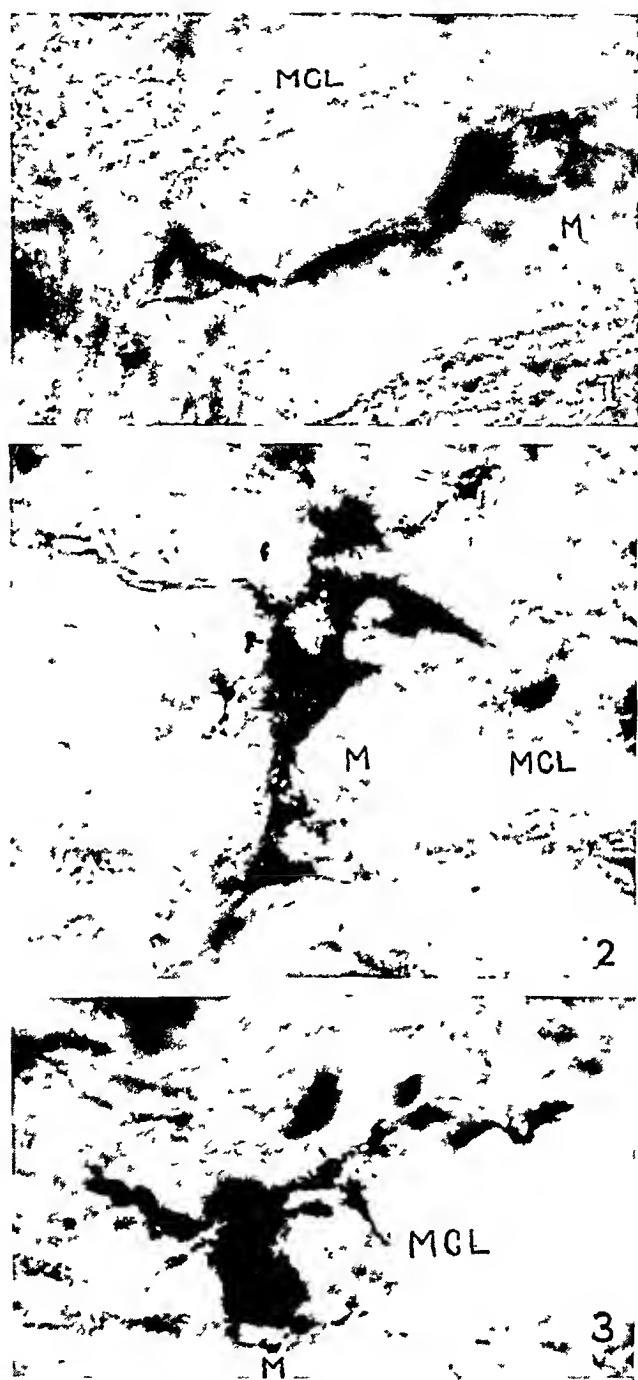


Fig. 11.—Secondary degeneration at ten days, showing hypertrophic microglia (*M*) attacking oligodendroglial myeloclasts (*MCL*); silver carbonate method of del Rio Hortega.



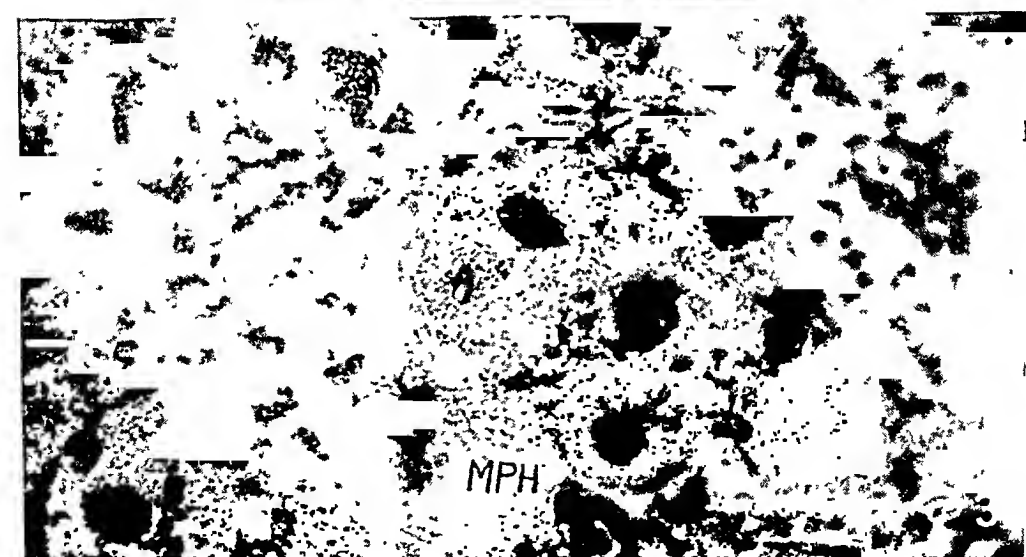
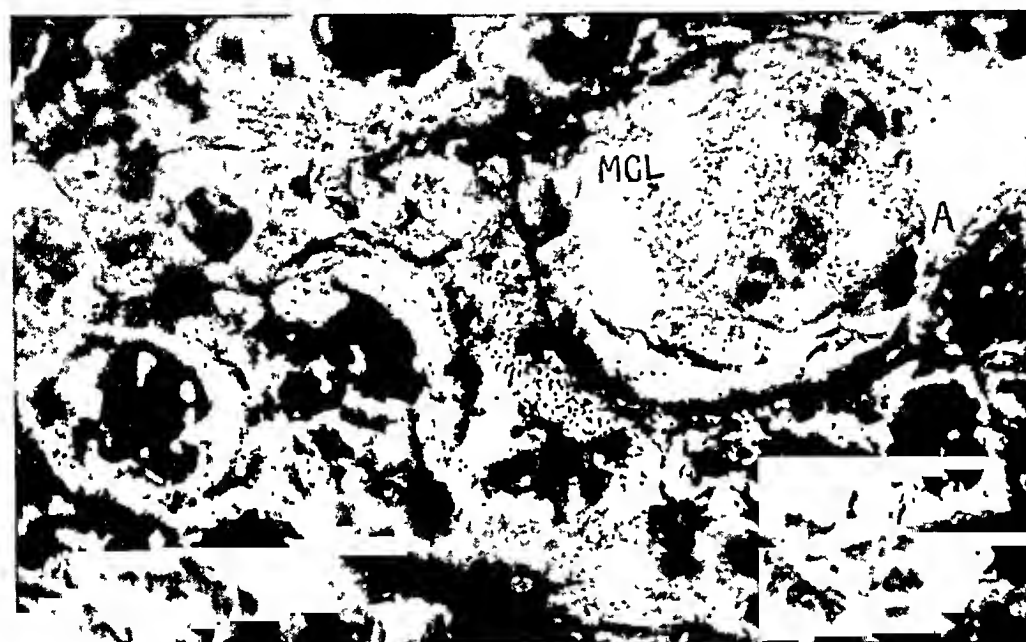
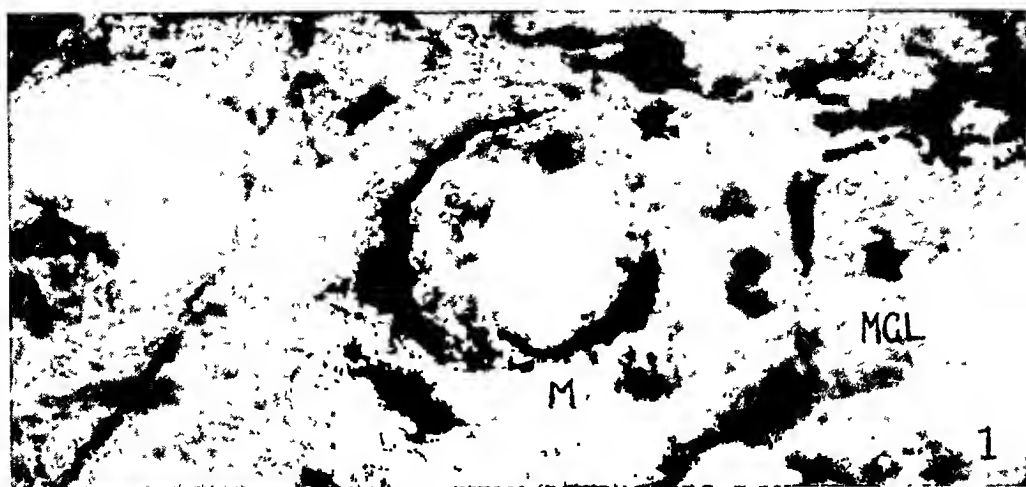


Fig. 12.—Secondary degeneration at twenty-four days: Section 1 shows microglia (*M*) about oligodendroglial myeloclasts (*MCL*); silver carbonate. Section 2 shows astrocytes (*A*) around myeloclasts (*MCL*) and section 3, oligodendroglial myelophages (*MPH*); gold sublimate method.

portation, and the two ectodermal elements will be shown to be remarkably active.

Meanwhile the myelophages continue with the penultimate phase of fat digestion, preparing for the final stage of digestion on the part of the fettkoernchen cells alpha. Some of the myelophages also are destroyed in this process; others doubtless remain intact and become fettkoernchen cells alpha also.

The fettkoernchen cells alpha, or gitter cells, are the active cells of the seventh and eighth weeks, i. e., of our fifty-second day stage. As

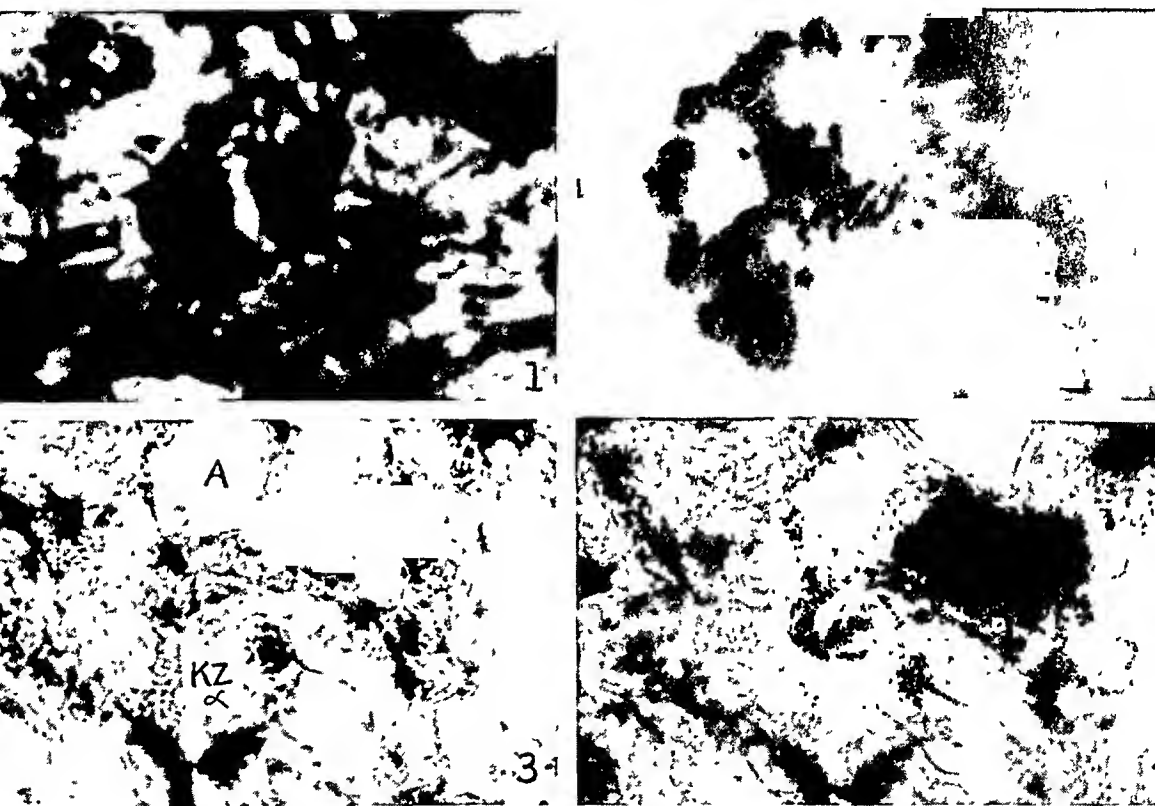


Fig. 13.—Secondary degeneration at fifty-two days, showing presence of fine fat in the fettkoernchen cells alpha ( $KZ\alpha$ ) and the beginning absorption of true fat from them by the astrocyte ( $A$ , section 3) or fettkoernchen cell beta. Section 1 represents the silver carbonate and Fett Ponceau stain; section 2, the same stain, showing the fat seen by polarized light. Section 3 shows a gold sublimate stain and Fett Ponceau, and section 4, a similar stain under polarized light.

intimated, they, too, are composed of oligodendroglia and microglia, as shown in figure 13. These cells, the forerunners of which were seen as the group of small cells in the thirty-fifth day stage which were once-removed in space and function from the myelophages, showed little tendency at that time toward activity. But meanwhile they have assumed the round, swollen, spokelike form and lie in coalesced groups.

Again, from the cyto-architectonic point of view, the differences between the numerous forms the ontogenetic forbears of which were oligodendrogliaocytes and the type that derived from the microglia can still be demonstrated. The former are globular and have a more or less centrally placed nucleus and a webbed, loculated cytoplasmic structure, which is seen to be filled with fat, when stained for this purpose. The type of fettkoernchen cells deriving from the microglia has occasionally the elongated form of these cells, or more frequently assumes a signet-ring form, with the nucleus lying eccentrically. These cells, too, can be demonstrated as filled with fat. Both types of cells have the common quality of a stable appearance. They have no longer the aspects of phagocytes, in the sense that the latter are a quickly reacting, in part degenerating and essentially mobile type of cell. Rather, they have the appearance of gland cells, and the ectodermal group in particular, i. e., the oligodendrogliaocytes, may be compared with digestive gland cells, both physically and functionally. First, they lie in clustered groups between the fibers of the macroglia supporting structures, as in figure 14—they themselves being now without their previous obvious fibrous connections with the glial and nervous structure. Secondly, even before they have become filled with fat, they have swollen as though elaborating a ferment for the expedition of the partially digested fatty stuffs that they absorb from their predecessors. Thirdly, it is within their bodies that the fat finally becomes neutralized, polarizable and capable of being transferred to the macroglia, *A* as illustrated in figure 15. Lastly, the cells that have been thus active do not seem to have been destroyed, for though their cell bodies are abnormally distended and their cytoplasm dispersed, so that they are with difficulty discerned in their entirety as cells, their nuclei have remained singularly intact. This allows for an interesting speculation, with the logical end that Penfield's presumption that the oligodendroglia are capable of regeneration after having undergone acute swelling is here made more obvious, perhaps, than in Penfield's own work with brain material.

Transportation is not the best descriptive term to be applied to the movement of the fat from the fettkoernchen cells alpha, i. e., the oligodendroglia and microglia, into the fettkoernchen cells beta, i. e., the macroglia; for that would imply a movement on the part of the vehicles themselves—the fettkoernchen cells. These cells, however, are only capable of passive mobility, and the movement of the fat is therefore to be thought of more as a flux—as a filtering phenomenon. The mobility of the small cells is accomplished by the gentle prehensile movements of the astrocytic prolongations, rather than as an automobility of the smaller, fat-laden cells. This is illustrated in figure 16. This introduces the macroglia as active elements in the final transpor-

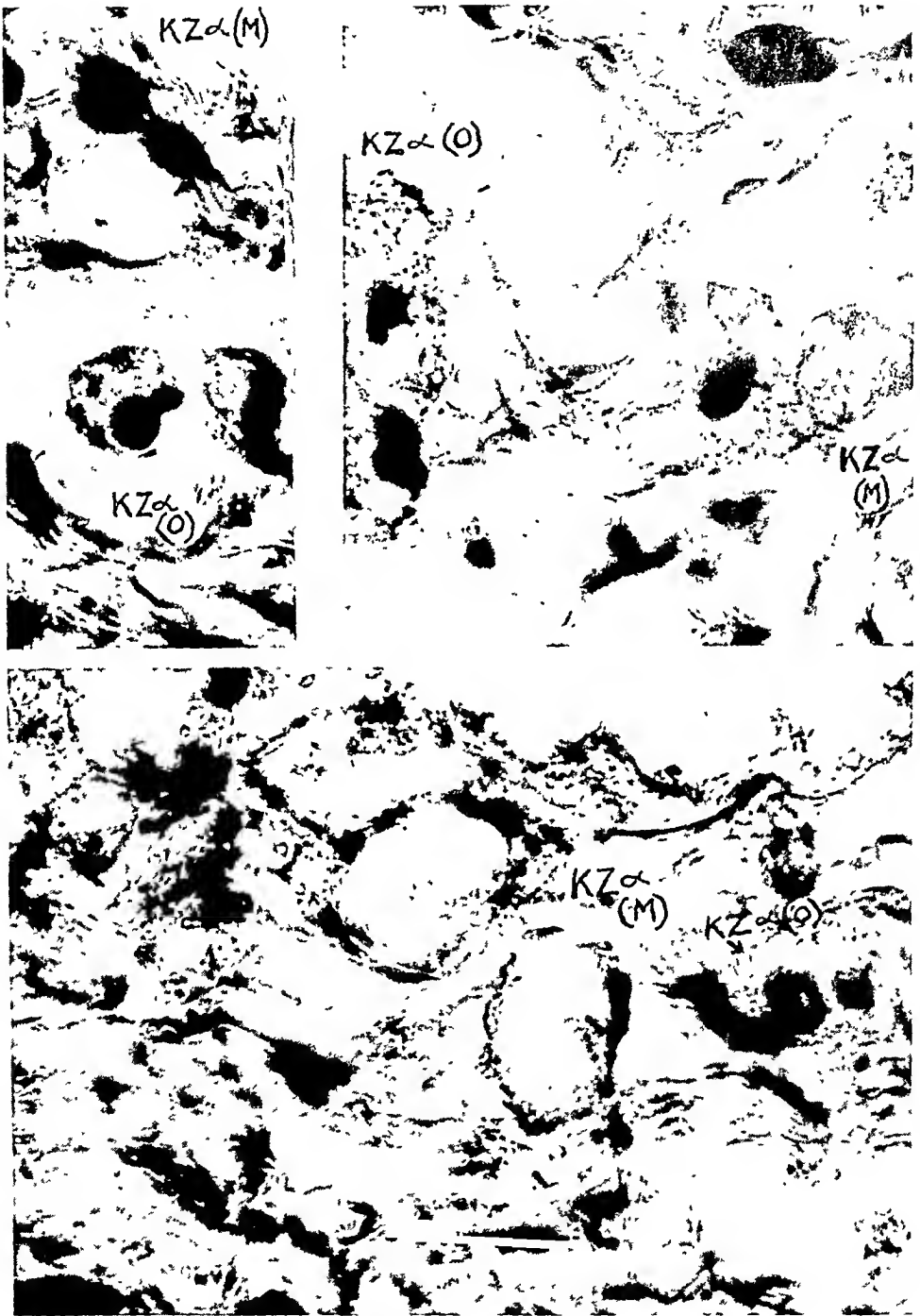


Fig. 14.—Secondary degeneration at fifty-two days, showing fettkoernchen cells alpha ( $KZ\alpha$ ) derived from oligodendroglia  $KZ\alpha$  (O) and microglia  $KZ\alpha$  (M), respectively; silver-carbonate method of del Rio Hortega.

tation of the fat from the tissues into the blood vessels of the spinal cord—their rôle as the so-called fettkoernchen cells beta.

*Macroglia in Late Stages.*—In this study, we have carried the investigation of the glial reactions to the secondary degeneration of the spinal cord fibers up to the seventy-eighth postoperative day, and have watched the transformation of the products of the degeneration of axis cylinders and myelin sheaths being changed, by the intercession of the oligodendroglia and the microglia, through the prelipoid stages into the final form of true fat, in which quality the degeneration products are lodged in the macroglia.

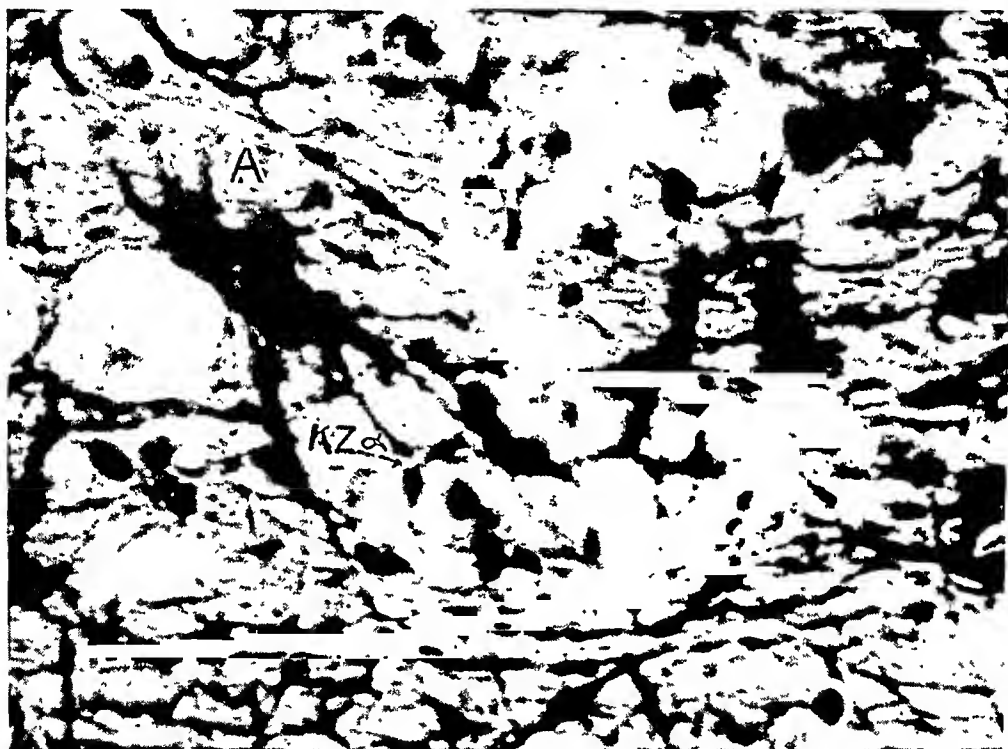


Fig. 15.—Secondary degeneration at fifty-two days, showing astrocytes (*A*) surrounding fettkoernchen cells alpha (*KZα*); gold sublimate method.

In résumé, the activities of the macroglia up to this time have been as follows: In the early stages, up to the fifth day, the macroglia react by hypertrophy and hyperplasia. Many examples of twin-nucleated or multinucleated macroglia can be observed. There is little evidence of clasmotodendrosis. At about the tenth day, the macroglia can be seen building strong fibrous rings about the degenerating axomyelin structure, in which the oligodendroglia, at first, and the microglia, secondarily, play an active phagocytic rôle, leading to their destruction within these first disintegrating masses of nerve tissue. In somewhat later stages, there is an intimation of a tendency on the part of the macroglia to

augment their number by forming new, protoplasmic-appearing cells and to show vacuolated areas within certain of the individual astrocytes. In the stage of the fifty-second day, the astrocytes are seen to surround the fettkoernchen cells alpha, which contain practically all of the true fat that has been formed up to this time. Their prolongments are seen to surround and to permeate the areas containing these small, fat-filled cells, and along the fine, flufflike prolongments of the astrocytes

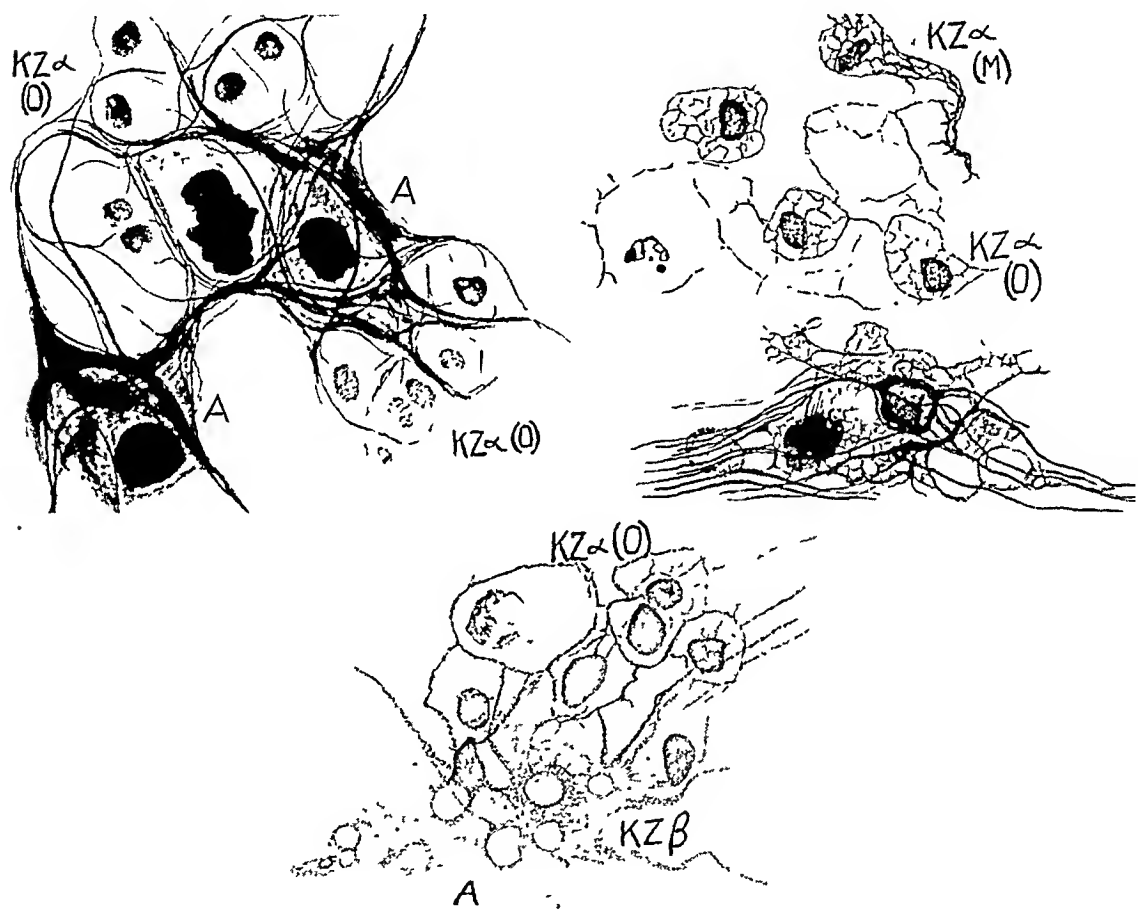


Fig. 16.—Secondary degeneration at fifty-two days, showing formation of fettkoernchen cells beta ( $KZ\beta$ ) by the absorption of astrocytes ( $A$ ) of the fettkoernchen cells alpha ( $KZ\alpha$  [O]) and ( $KZ\alpha$  [M]), which have been derived from oligodendroglia and microglia.

the fat can be seen in tiny, elongated, crystalloid forms entering into these extensions of the astrocytic body. In the larger portions of these prolongations of the macroglia, one can see, extremely frequently, the nuclei of the fettkoernchen cells being engulfed by the macroglia and contained within vacuoles of the latter.

Between the fifty-second and the seventy-eighth day, the migration of the fat from the small fettkoernchen cells into the macroglia is prac-

tically completed. Whereas in the previous stage there were only occasional macroglia that showed globules of fat within the cytoplasm, the latest stage shows that nearly all of the fat has been thus transferred, and it is now only occasionally that the smaller cells contain much of the fat. An added phenomenon of interest is that many of the small fettkoernchen cells, with their load of fat, have been completely encompassed within the larger macroglia. Because these cells, thus engulfed, with their nuclei surrounded by scarlet-staining fat that is double-polarizing, still retain, in their intramacroglial berths, the essential appearance that they had while only surrounded by the macroglia, it is believed that they continue to exert their fermentative functions in this new location. That is to say, the macroglia, as we have observed them in their fat-absorbing capacity, seem incapable of prehending prelipoids; for the most part, theyprehend only the already prepared fat. But when they absorb incompletely digested fatty material, it is frequently still accompanied by the glandlike small cells that have been the only active phagocytes until this time. We were unable to carry the investigation to such a stage as would show the ultimate disposal of these absorbed cells. Figure 17 shows macroglia filled with fat; section 1 of the same figure shows similar fat-filled astrocytes attached to a blood vessel.

As a final intimation as to the manner of the disposal of the fat thus absorbed by the macroglia are the preparations that show the astrocytes attached, with their fat-filled protoplasmic prolongations, to the blood vessels. Our investigations do not allow us to describe the further changes in the intramacroglial fat leading to its ultimate discharge into the lymph and blood vascular system, as Jakob described this disposition in his work, which covers a much longer period of time. Furthermore, we are unable to state the exact nature of the fettkoernchen cells beta. We have used this term because it was used by Jakob in his original work on secondary degeneration. Whether these cells actually act as gitter cells cannot be stated definitely here because our investigations were not carried far enough to determine this definitely. We know that these cells encircle and engulf polarizable fat from the fettkoernchen cells alpha, and that they show such fat within their cell bodies. What the exact significance of this activity is, we prefer to leave an open question for the moment.

Another problem as yet unsolved in the scope of our experiments is the function of certain new, protoplasmic astrocytes that are seen abundantly in this stage. These are large, polygonal astrocytic structures with round, darkly impregnating, finely granular nuclei and a paler, more coarsely granular cytoplasm, extending into typical macroglial prolongments. They are, for the most part, nonfibrous, although they contain fiber-like forms within their prolongments. Morpho-

logically, they resemble the swollen forms of oligodendroglia, and many of the smaller cells cannot be differentiated from the latter.

As to the origin of these new astrocytes, we cite two possibilities, both of which seem tenable on the basis of our observations.

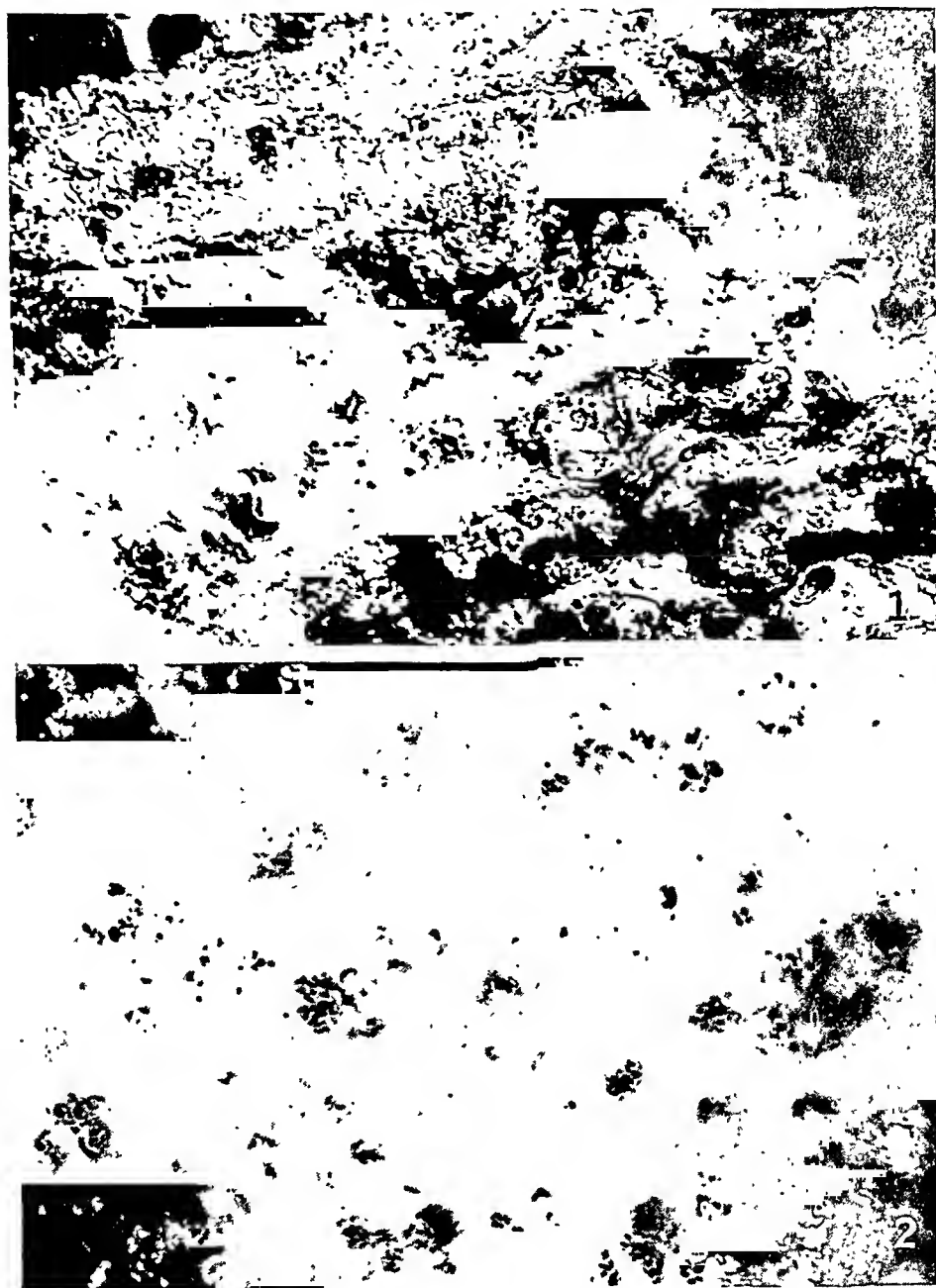


Fig. 17.—Secondary degeneration at seventy-eight days: Fat-filled astrocytes attached to blood vessels seen in ordinary light (section 1) and in polarized light (section 2); gold sublimate-Fett Ponceau.

The first is that they are derived by amitotic division of normal, older astrocytes. We have seen many examples of such division and



have discussed the phenomenon in an earlier paragraph dealing with the changes observed in the second week of the degeneration.

The second possible source is from the new, small, round cells that arise in such great numbers about the end of the second week of the

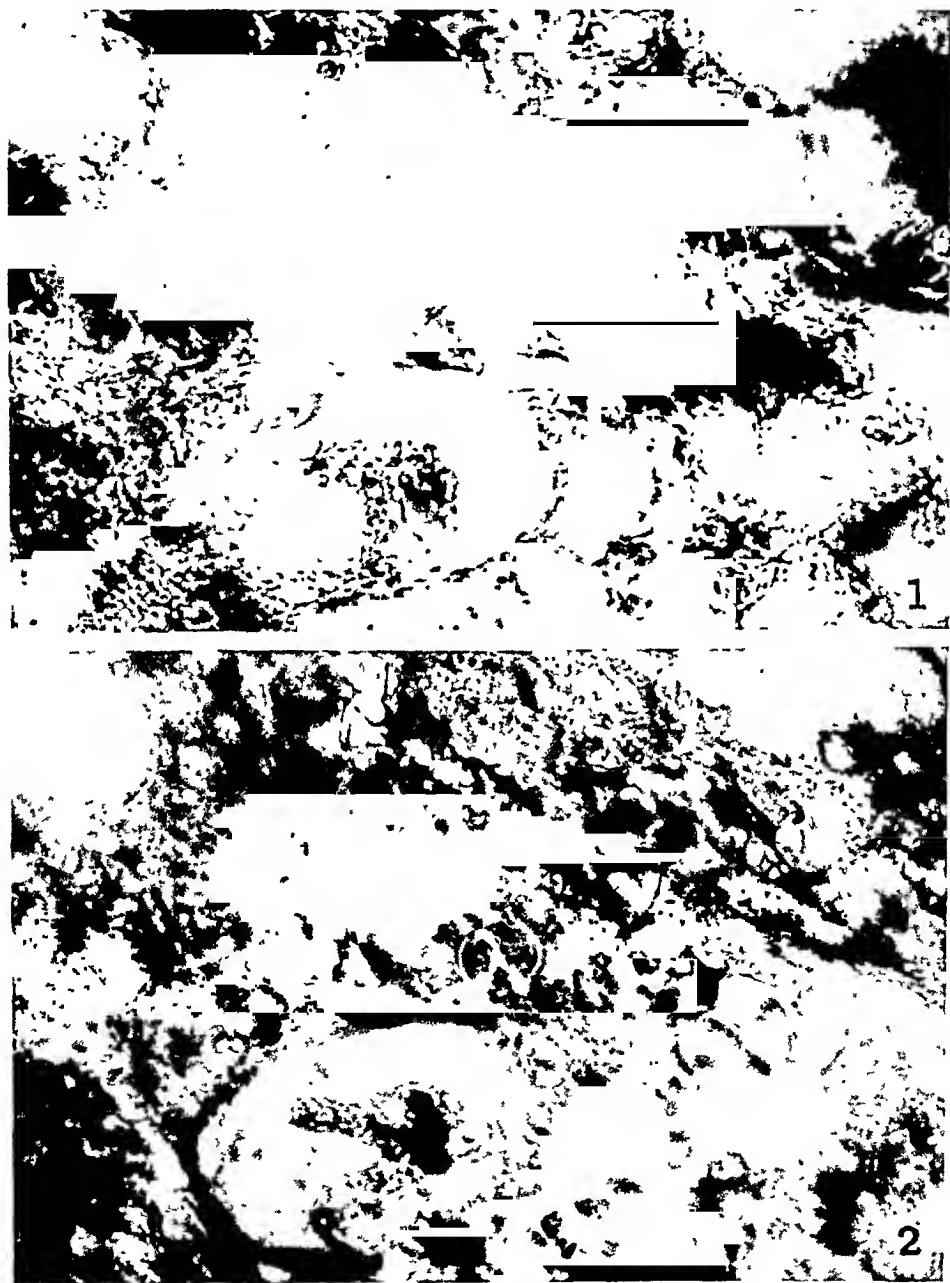


Fig. 18.—Secondary degeneration at seventy-eight days, showing a giant astrocyte filled with fat droplets that photograph black by ordinary light, but are permeable by polarized light (section 2), in which they are seen as globules containing maltese crosses; gold sublimate-Fett Ponceau.

degeneration. These cells are rather undifferentiated and for the most part resemble oligodendroglia. We believe that they are purely ectodermal and give rise to oligodendroglia and astrocytes. They impreg-

nate with gold sublimate and with silver carbonate equally well, and no differentiation can be made between the ones that are acting as genitors of new oligodendroglia and those that are acting as genitors of macroglia, until the later stages. In figure 19, section 1, representing the seventy-eighth day stage of degeneration, the final attainment in the

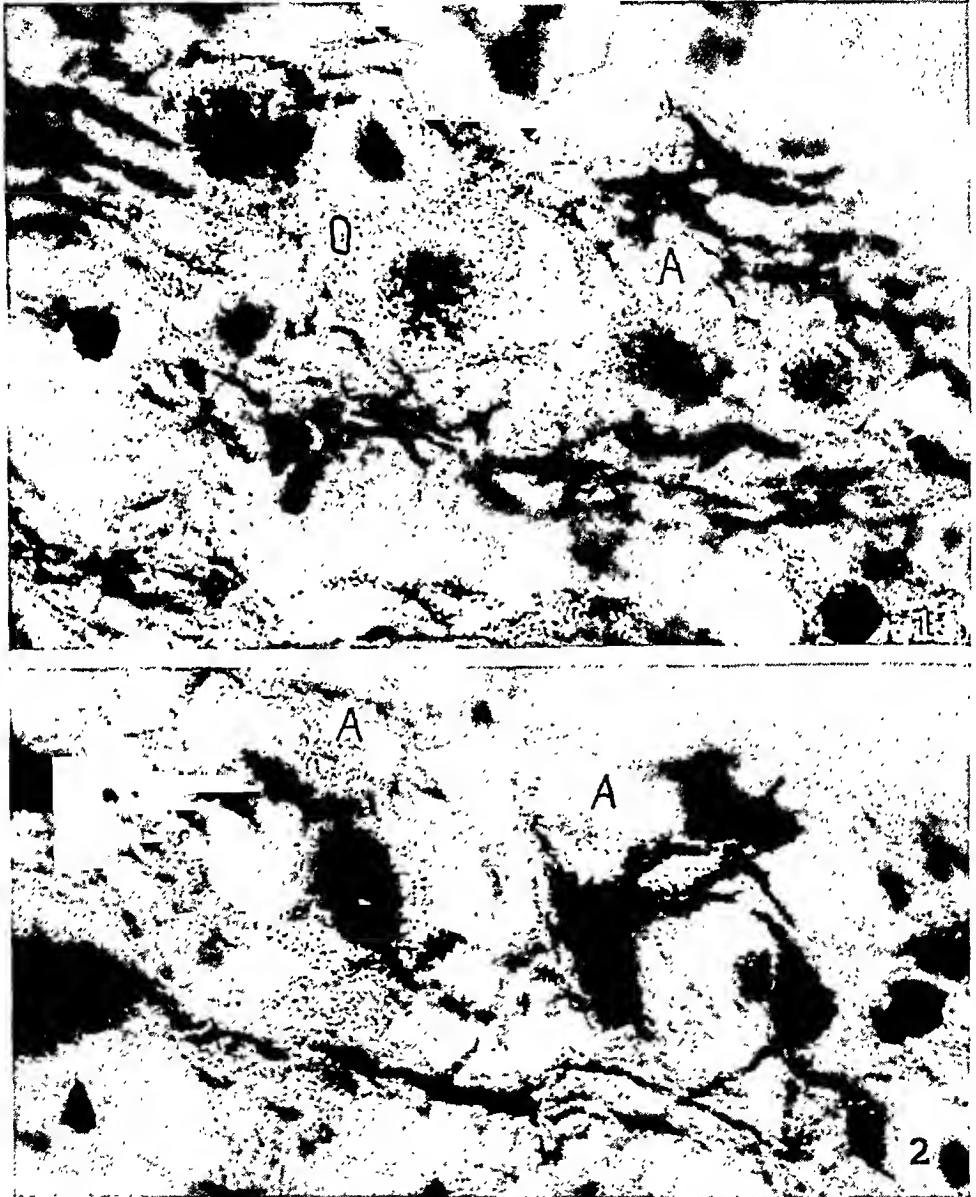


Fig. 19.—Secondary degeneration at seventy-eight days, showing new, protoplasmic astrocytes (*A*) arising in the field of recent secondary degeneration.

size and the shape of the differentiating characters is shown. We consider the function of these new astrocytes to be twofold: (1) secretory-digestive and (2) supportive.

We attribute the secretory-digestive function to them on the basis of the complete loss of normality on the part of the older astrocytes,

which have been acting as fettkoernchen cells beta. For in the latter, one sees an almost complete replacement of the body substance by fat droplets absorbed from the adjacent fettkoernchen cells alpha. The astrocytes become mere webs of tissue and seem to have succumbed in this process of engorgement. It is also probable that this passage of fat through the astrocytes is not unaccompanied by a secretive-digestive activity by these ectodermal, and therefore possibly glandular, cells. Inasmuch as we have seen, from time to time, fat inclusions within these new, protoplasmic astrocytes, we regard them as cells that have developed in order to reabsorb and more completely digest the fat resting within astrocytes that have exhausted their digestive capacities as fettkoernchen cells beta.

Aside from this active glandlike function of the new astrocytes is the simpler function of merely replacing their predecessors in performing the normal duties of support and nutrition. For it is true, as we shall show immediately, that having at this stage divulged themselves of the greater part of their burden of fat, these astrocytes proceed to become part of a dense macroglial cicatrix.

In the old, fibrous macroglia there is now a definite tendency to cicatrix formation. Their strongly impregnated fibrous prolongments surround the old rings in which the preliminary digestion of fat took place, and these rings are apparently empty, except for the nuclei of the fettkoernchen cells that have given up their fat. In marked contrast to the strips of normal tissue that escaped the degenerative process, in which the intact axis cylinders and their lightly impregnated myelin sheaths are seen lying within the meshes of the astrocytes, and the rows of normal oligodendroglia, are these rings built of the deeply impregnating and strongly fibrous macroglia.

#### SUMMARY

A study of experimental secondary degeneration of the fibers of the spinal cord in rabbits was made, with the use of the metallic impregnation methods of the Spanish school, the aim being to study the three types of glia in this reaction.

The degenerative reactions were studied in various representative stages up to and including the seventy-eighth postoperative day.

The study revealed and confirmed the fact that the glial cells, both ectodermal and mesodermal, were the active agents in the phagocytosis of the degenerated nerve fibers.

The oligodendroglia were shown to be the first active glial cells in the phagocytosis of the effete nerve fibers.

In conjunction with a belated activity on the part of the microglia, the oligodendroglia were shown to continue their phagocytic activities until the axomyeline material had been fermented into true fat.

The substantiation of the fact of active phagocytosis on the part of the oligodendroglia, accompanied by that of the microglia, was made on the basis of distinctly different cyto-architectonic qualities in the phagocytic derivatives of these two types of cells.

The phagocytic-digestive activity of the ectodermal types, i. e., the oligodendroglia, is compared to that of glandular cells.

The astrocytes were shown to have a particularly sturdy reaction during the earlier weeks of the degenerative process, with some exceptions, but in the later weeks, to engulf fat and the other products of the degenerated nerve fibers.

A hyperplasia on the part of the ectodermal glia, leading to the formation of new oligodendroglia and macroglia presumably for the purposes of the ultimate digestion and disposition of the fat, was demonstrated.

The last function of the macroglia was seen to be in the nature of cicatrix formation in the areas of degeneration.

### CONCLUSIONS

By the use of a purely experimental means of investigation and the avoidance thus of the complications presented by postmortem changes, the activities of the glia in secondary degeneration of the fibers of the spinal cord, and particularly the functions of the very susceptible oligodendroglia, can be successfully studied. The impregnation methods of the Spanish school constitute a satisfactory means of differentiating between the various types of glia that function as gitter cells.

The oligodendroglia are the elementary glial units in this neuropathologic process, being the first cells to function as myeloclasts. Later they work in similar capacities and as myelophages in conjunction with microglia.

The microglia, always much more sparse in the white matter of the spinal cord than in other parts of the central nervous system, play the rôle of secondary invaders of the seat of phagocytosis. They are mobilized and drawn into this seat of action from a distance. After the primary myeloclastic work of the oligodendroglia and the secondary myeloclastic activities of the oligodendroglia and the microglia working in conjunction, new groups of these two types of glia act together to become myelophages, and others later function as fettkoernchen cells.

The astrocytes act as purely supportive cells in the earlier periods of the degeneration. As the digestive activities of the smaller cells become productive of true fat, the latter is gradually absorbed into the surrounding macroglia.

There are a permanent hyperplasia and a regeneration of the ectodermal elements, forming new oligodendroglia and macroglia.

# CHLORO-ERYTHROBLASTOMA

## REPORT OF A CASE \*

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The relationship of chloroma to the leukemic diseases is so well established, its clinical features and its pathologic lesions so well known, that the report of a single case is hardly justified unless the study presents some new fact in addition to those generally known.

The presence of large numbers of megaloblasts and normoblasts, primitive forerunners of the erythrocytes, in an otherwise typical case of myeloid chloroma seemed sufficiently interesting to warrant recording this case under the descriptive term of chloro-erythroblastoma.

Dock,<sup>1</sup> while studying the relationship of chloroma to the clinical observations in the blood, pointed out the similarity between the blood cells in chloroma and those in leukemia. Studies by Dock and Warthin<sup>2</sup> further pointed out the general systemic involvement in chloroma and definitely established the relationship of chloroma to leukemia. From morphologic studies of the cells in chloromatous tumors, they have been classified into lymphoid and myeloid types. However, Brannon<sup>3</sup> in an excellent review stated that the cases reported as belonging to the lymphoid type lack complete study of the tumor, bone marrow and blood smears, especially with reference to the peroxidase reaction. This he feels is necessary to establish the diagnosis of a lymphoid chloroma, and he agrees with Burgess<sup>4</sup> and Askanazy<sup>5</sup> that chloromas are always myeloid in origin.

## REPORT OF CASE

*History.*—A previously healthy girl, aged 6¾ years, was admitted to the San Francisco Hospital because of a prominence of the left eye. Eight weeks prior to admission, her mother noticed that the child's left upper eyelid drooped. Four weeks before entry, the eyeball appeared to protrude forward, and the upper lid became swollen. Vision apparently was undisturbed, and there was no pain.

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\* From the Departments of Surgery and Pathology, Stanford University Medical School.

1. Dock, George: *Am. J. M. Sc.* **106**:152, 1893.

2. Dock, George, and Warthin, A. S.: *Tr. A. Am. Physicians* **19**:64, 1904.

3. Brannon, Dorsey: *Bull. Johns Hopkins Hosp.* **38**:189, 1926.

4. Burgess, A. M.: *J. M. Research* **22**:133, 1912-1913.

5. Askanazy, M.: *Beitr. z. path. Anat. u. z. allg. Path.* **63**:22, 1916-1917.

There was no history of familial diseases. Both parents were healthy. The child was born at full term, with spontaneous delivery. She was breast fed, and had normal growth and development. Chickenpox was the only disease of childhood contracted by the patient.

*Physical Examination.*—On admission, the rectal temperature was 99 F., the pulse rate 120 and the respiratory rate 22. The child did not appear ill and was well nourished. The left side of the head was prominently bosselated. The ears were normal. The left eye showed swelling of the upper lid with distention of the veins. There was proptosis with drooping of the eyelid, and the eyeball was displaced downward and outward by a mass in the upper nasal quadrant of the orbit. The tumor appeared about 1 by 2 cm. in size, and was located just below the supra-orbital ridge, and was apparently attached to the roof of the orbit. It was firm and elastic and had a smooth rounded edge. Proper convergence was prevented by the proptosis and outward displacement of the eyeball. The movements of the eyeball were all limited, especially the action caused by the superior rectus and superior oblique. The pupils were regular, reacted to light and in accommodation, and the left pupil was slightly larger than the right. Examination of the optic fundi showed dilatation of the veins on the left, with some blurring of the nasal half of the optic disk.

The head and neck were otherwise normal. The cranial nerves were normal with the exception of the movements of the eyeball as noted. The chest, heart and lungs revealed no abnormalities. The edge of the liver was felt just below the costal margin, and the tip of the spleen was barely palpable. The reflexes of the extremities were all normal. A roentgenogram of the skull revealed no defects in bony structure.

The urine showed no abnormalities. No attempt was made to detect the presence of the Bence-Jones protein. The blood revealed 3,000,000 erythrocytes per cubic millimeter, with a hemoglobin content of 60 per cent (Sahli); the white cells numbered 17,650, of which 66 per cent were polymorphonuclear cells and 34 per cent mononuclear cells, interpreted as being lymphocytes.

Because of the rapid growth after the onset of the symptoms, Dr. Leo Eloesser thought the tumor was malignant, probably a chloroma of the orbit.

*Therapy.*—On Nov. 28, 1930, the child was given preoperative roentgen therapy over the left orbit by Dr. R. R. Newell. The next morning the eyelids were more swollen and reddened in the area of irradiation. The same day Dr. Eloesser operated on the patient. The orbit was exposed by a modified Kroenlein incision; an incision parallel to the eyebrow and reaching the nasal sulcus was added, to give adequate exposure of the inner half of the orbit. On splitting the retro-ocular fascia, the tumor was seen to be lobulated and encapsulated, but it extended into the rear of the orbit, so that the zygoma was doubly incised and displaced outward to allow an adequate approach to the tumor, which was attached to the periosteum at the inner surface of the orbit. Here it was removed by galvanocautery, with a portion of the periosteum and a thin slice of the underlying bone. The wound was closed and drained by a small strip of rubber tissue. The eyeball and extra-ocular muscles were preserved.

The convalescence was uneventful. Roentgenograms of the skull, chest and long bones showed no bony abnormalities or metastatic deposits. The blood, Dec. 6, 1930, showed an erythrocyte count of 3,000,000, a hemoglobin content of 59 per cent (Sahli), and a white cell count of 21,050, of which 59.3 per cent were polymorphonuclear leukocytes, 26 per cent lymphocytes, 3.3 per cent large mononuclears, 0.3 per cent basophils, 8 per cent neutrophil myelocytes and 3.3 per cent myeloblasts. The red cells varied in size and shape. There were large erythrocytes with polychromasia, and two megaloblasts were seen.

Under the direction of Dr. R. R. Newell, a postoperative course of deep roentgen therapy was begun, the plan being to give small filtered doses over the entire body, with exposures twice weekly.

The observations on the blood are shown in the table.

*Examination of Removed Tumor.*—The tumor measured  $3\frac{1}{2}$  by 3 by  $1\frac{1}{2}$  cm. The mass had one smooth, flat surface, while the opposite side presented a lobulated appearance similar to that of pancreatic tissue. The tumor was enclosed in a thin, transparent capsule, through which the tissue appeared as an olive green mass. The color was the most striking quality of the specimen. Section showed

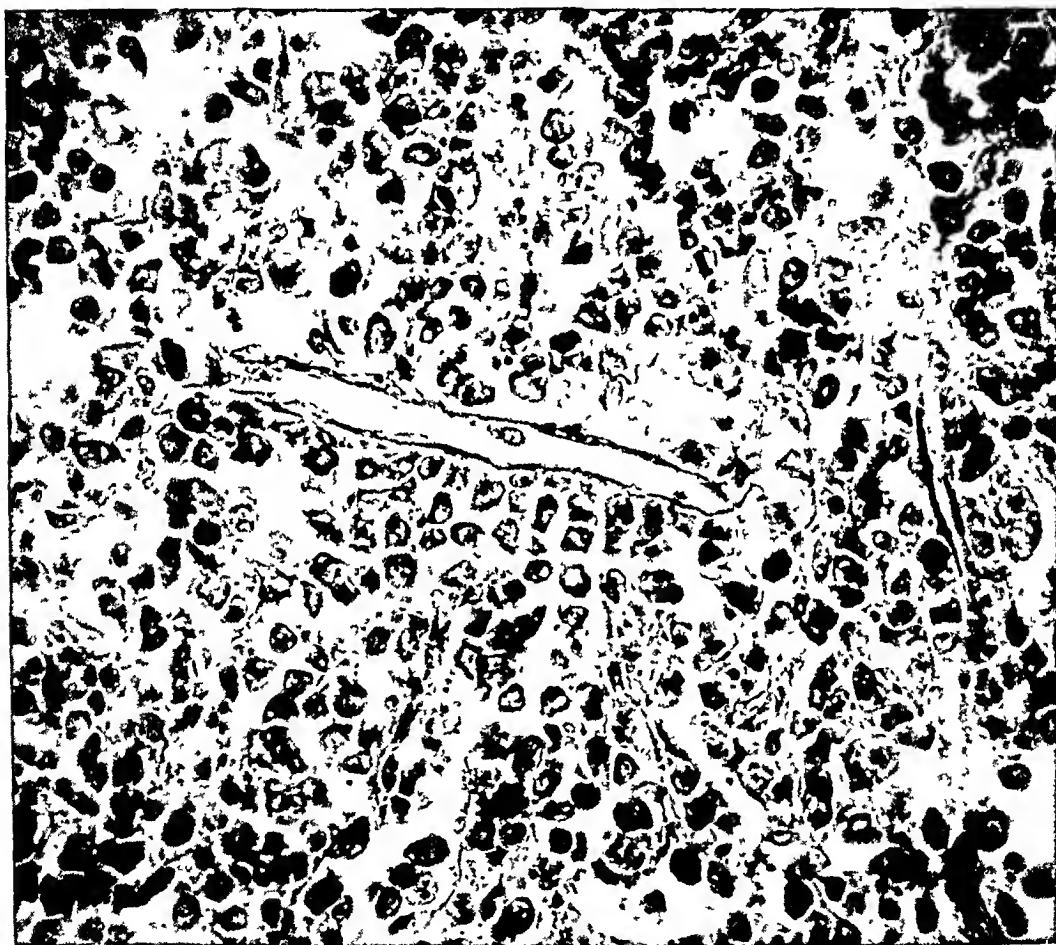


Fig. 1.—A low power photomicrograph showing the general architecture of the chloroma. The myeloid cells are in close approximation and parallel to the thin-walled capillaries. In the central vessel is shown the detachment of a swollen endothelial cell from the wall into the lumen.

no definite structure. The tissue was homogeneous, firm and elastic. The color faded to a dirty gray-green within one hour, but was restored, even three months later, by placing the tumor in hydrogen peroxide.

Microscopic paraffin sections stained with Giemsa's stain showed the tumor to be composed of closely packed cells arranged definitely parallel to thin-walled capillaries and blood sinuses, the architecture of the tumor being apparently dependent on the vascular arrangement (fig. 1). There was a fine, indistinct reticulum loosely binding the cells outside the walls of the blood vessels. The

type of cell most frequently encountered had a large, rounded or slightly oval vesicular nucleus with a surrounding thin investment of basophil cytoplasm, agranular and without vacuoles. These cells corresponded to the myeloblasts of normal bone marrow. Numerous myelocytes were seen with round or slightly indented nuclei and with granular cytoplasm. Those with eosinophil granules predominated, while the neutrophil myelocytes were less numerous. Only a very occasional basophil granular cell was encountered. While polymorphonuclear leukocytes were found, most of them lay within the lumen of the blood vessels. Rendered distinct by their dark, pyknotic nuclei, the cells of the erythrocytic series were found throughout the microscopic section (fig. 2). Megaloblasts with

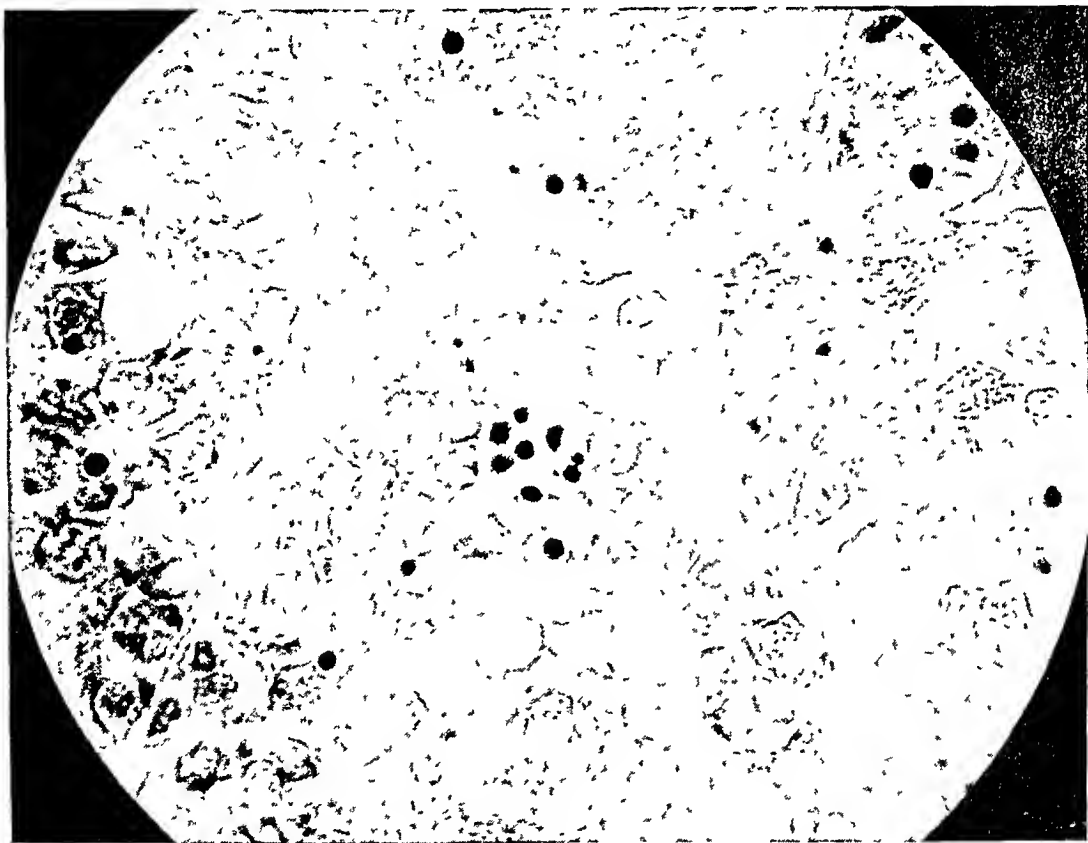


Fig. 2.—A high power photomicrograph in which groups of erythroblasts and megaloblasts are rendered distinct by the contrast of their small, pyknotic nuclei with the larger, faintly granular nuclei of the myeloblastic cells. With the Giemsa stain, the cytoplasm of the erythroblastic cells shows varying stages of accumulation of the eosinophil hemoglobin.

basophil, agranular, clear, pyknotic nuclei, frequently placed eccentrically, composed the majority of the cells of the erythrocytic type. One could see many of the stages in the maturation of the red cell: megaloblasts with beginning polychromasia, normoblasts with a full complement of hemoglobin and with small, dark, pyknotic nuclei, which occasionally showed karyorrhexis—all these types were encountered. Howell-Jolly bodies were occasionally seen in the erythrocytes lying outside the vascular wall, and macrocytes with polychromasia were frequently seen.



Many of the endothelial cells of the thin-walled capillaries and sinuses appeared swollen and rounded (figs. 3 and 4). In longitudinal profile of the vessel, this was seen to take place in isolated sections of the wall of the blood vessel, while normal, thin, spindle-shaped endothelial cells were interspersed between the rounded cells. These rounded endothelial cells might bulge into the lumen or to the outside of the vessel, then occupying a position among and almost

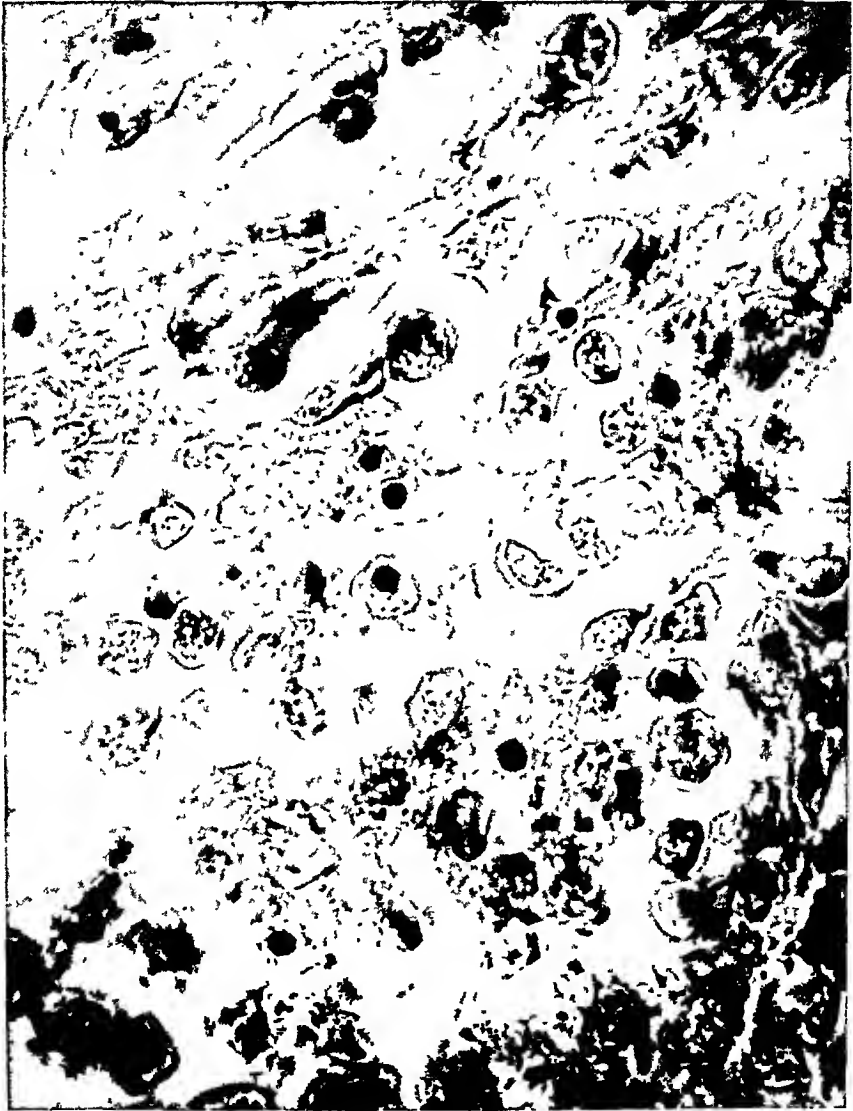


Fig. 3.—A high power photomicrograph. At the top of the field are blood sinusoids showing rounding up and stages of detachment of the endothelial cells from the walls of the vessel into the lumen. Many cells of the erythrocytic series are seen scattered among the myeloid cells.

surrounded by the closely packed myeloid cells. The rounded endothelial cells had changed staining characteristics as well; the cytoplasm was more distinct and basophil, and the nucleus more rounded and darker, although still vesicular. The long, oval, branched reticulum cells, with indistinct cytoplasmic outlines, occasionally were seen to undergo a similar transformation, with a rounding up of the cytoplasm, which became more distinct and basophil, while the nucleus became

more rounded with a more distinct chromatin network. No megakaryocytes or blood platelets were found among the tumor cells. Lymphocytes and plasma cells were likewise absent. Mitotic figures were seldom seen. With the peroxidase reaction, the cells described as myeloblasts, myelocytes and polymorphonuclear leukocytes showed distinct granules of the peroxidase type. No pigment, either amorphous, extracellular or intracellular, could be seen in fresh or permanent sections. No crystals similar to Charcot's crystals were found such as those in the chloroma described by Askanazy.

*Subsequent Course.*—The child was admitted to the Stanford Hospital on Feb. 13, 1931, because of profuse epistaxis beginning that morning. Two weeks before,

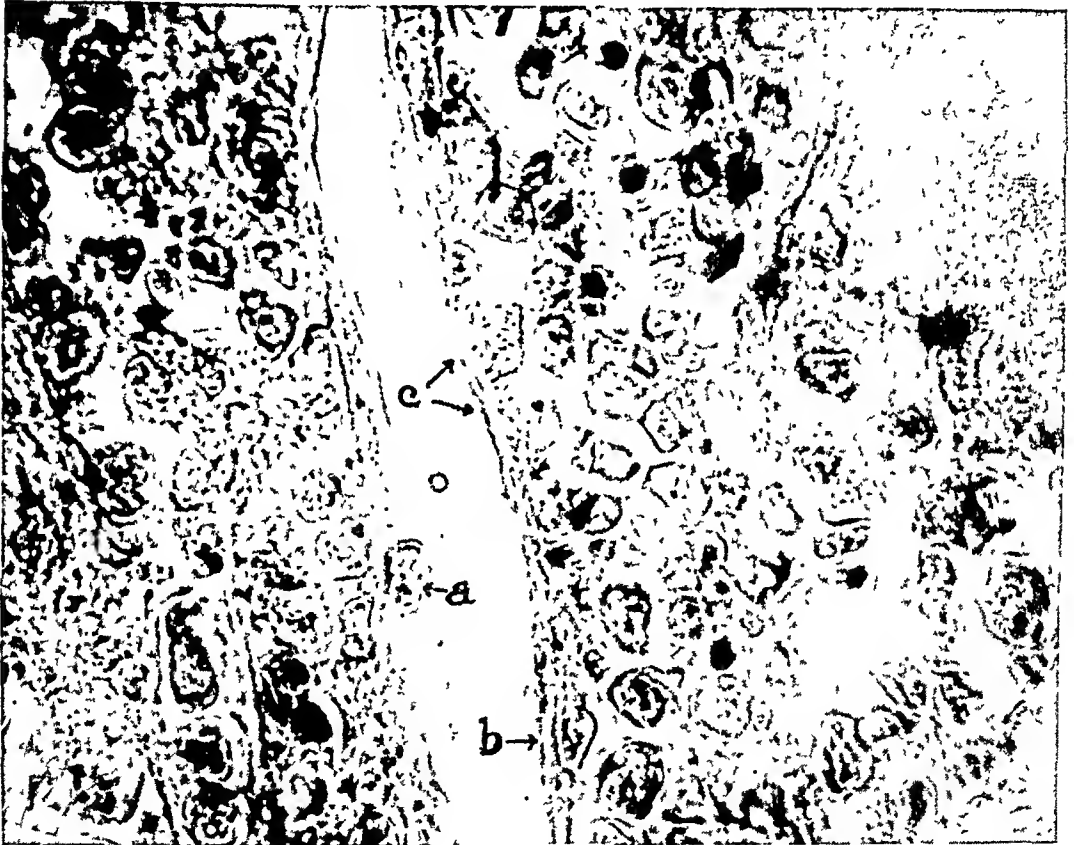


Fig. 4.—A high power photomicrograph. The small blood vessel running through the center of the field shows rounding up of the endothelial cells of the wall of the vessel, with protrusion into the lumen at *a*, and into the surrounding tumor at *b* and *c*. Myeloid cells predominate, but megaloblasts are present, recognized easily by the small, pyknotic, eccentric nucleus.

the mother had noticed that the child bruised easily. Four days before, a dull pain developed in the patient's left groin, which had persisted.

Examination revealed an apathetic, extremely pale and ill-looking young girl, bleeding from the left nostril. The rectal temperature was 100.8 F., and the pulse rate, 128. The region of the left eye showed the operative scar, with drooping of the upper lid and protrusion of the eyeball. There were large ecchymoses in the left pectoral region and over the right calf, with petechial hemorrhages on the

lips and gums. The heart, lungs and abdomen failed to reveal any abnormalities, except tenderness in the left groin. Neurologic examination revealed nothing abnormal.

The urine showed a trace of albumin with an occasional red blood cell, but tests for the Bence-Jones protein gave negative results. The stool was markedly positive for occult blood. A series of roentgenograms of the skull revealed nothing other than thickening of the mucous membrane of the antrums. For the observations on the blood see the table.

Three days after entry, the child became stuporous, her temperature rose to 104 F., and her pulse rate was 160. The bulging of the left eyeball increased. She had marked air hunger, and there were loud, crackling râles throughout the chest. The child expired on the sixth day after entry.

*Autopsy.*—The body was that of a well developed, well nourished, pale, white girl, with areas of purplish discoloration of the skin variously distributed over

*Tabulated Observations in Blood in a Case of Chloro-Erythroblastoma*

Observation	Date						
	11/25/30	12/6/30	1/2/31	1/13/31	1/23/31	2/14/31	2/16/31
Red blood cells, millions.....	3	3	3.13	2.15	3.23	1.13	1.07
Hemoglobin, per cent (Sahli)....	60	59	55	55	55	23	20
Color index.....	1	0.98	0.83	1.3	0.85	1.04	1
Normoblasts.....	..	1	..	1	1	..	..
Megaloblasts.....	..	2	..	..	..	..	1
Polychromasia.....	..	+	++	++	..	++	..
Macrocytosis.....	..	+	..	..	..	+	..
Reticuloeytes, per cent.....	..	1	3.4	3.6	..	1	1.4
Stippling.....	..	..	+	..	..	..	..
Diameters, mm. ....	..	..	7.2	7.64	..	..	..
Platelets.....	..	..	118,940	75,000	..	1,000	4,000
Bleeding time.....	..	Normal	Normal	..	..	..	..
White blood cells.....	17,650	21,050	28,000	17,700	9,050	21,300	22,900
Polymorphonuclears, per cent...	66	59.3	32	51	55	27	25
Lymphocytes, per cent.....	34	26	36	33	37	16	10
Monoeytes, per cent.....	..	3.3	2	10	5	2	..
Eosinophils, per cent.....	..	..	..	..	1	1	..
Basophils, per cent.....	..	0.3	..	1	..	..	..
Myelocytes, per cent.....	..	8	28	4	2	13	22
Myeloblasts, per cent.....	..	3.3	2	1	..	41	43

the body. A healed incision followed the left eyebrow and extended laterally for several centimeters. The left eye was markedly protuberant with congestion of the cornea and iris. Both pupils were equally dilated. The lymph nodes were not palpable. There was a very slight edema of the lower extremities.

The surface of the scalp next to the calvarium contained many moderate-sized hemorrhagic areas. The calvarium was normal, with moderate prominence of the vascular markings. The dura contained many small, flat, green, thickened areas averaging 1 cm. in diameter, rather uniformly distributed. The falx and tentorium were normal. There was slight flattening of the cerebral convolutions, with moderate congestion of the blood vessels. The cerebrospinal fluid was clear and normal in quantity. Many small hemorrhages covered the cerebellum. There was an olive-green, moderately firm, elastic tumor, measuring  $2\frac{1}{2}$  by  $2\frac{1}{2}$  by 2 cm., firmly adherent to the dura and to the bone beneath, extending into the left occipital lobe of the brain but only loosely attached to it (fig. 5). The bone beneath the tumor was eroded and contained numerous small, frostlike spicules extending into the dura and tumor. The pia-arachnoid space for 2 to 3 cm. surrounding the occipital brain defect was green. The hypophysis appeared normal. There was a green color visible through both orbital plates, the bones of

the middle cranial fossae and the sella turcica of the sphenoid. When the orbital plates were removed, masses of olive-green tumor were found in both orbits, chiefly superiorly, with none beneath and none penetrating the eyeballs. The marrow of the sella turcica and temporal bones was olive-green. The venous sinuses contained fluid blood and postmortem clots.

In the thoracic cavity there was a flat, olive-green mass, measuring 2 by 1.5 by 0.5 cm., firmly attached beneath the manubrium of the sternum. The spongy marrow of the sternum was brownish green.

The left pleural cavity was partially obliterated by adhesions of the visceral pleura to a green tumor measuring 3 by 2.5 by 1.5 cm., firmly attached to the third and fourth ribs posteriorly. The right pleural cavity was free from

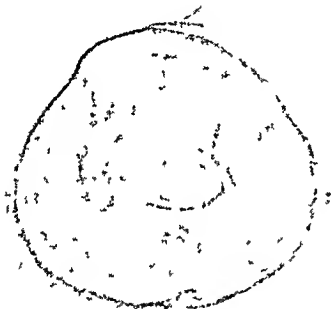


Fig. 5—A drawing of chloromatous nodules in the dura removed at autopsy. The large tumor protruded into the left occipital lobe of the brain and was firmly adherent to the skull.

adhesions, but there was a small, green nodule, measuring 1 cm. in diameter, adherent to the sixth rib near the vertebra. The pericardium was smooth and contained a few milliliters of clear fluid.

The heart weighed 150 Gm. The external appearance was normal, except for numerous small epicardial hemorrhages. The chambers, valves, coronary arteries and aorta were normal. The foramen ovale and ductus arteriosus were closed. Small hemorrhagic areas were distributed throughout the ventricular muscle.

The pleura of the left lung was smooth, except for the area attached to the tumor and a moderate number of small hemorrhages. The cut surface of the lung contained many scattered hemorrhagic areas of consolidation and exuded a

large amount of edematous fluid. The tumor appeared to be in the pleura only and did not penetrate into the lung. The bronchi contained a small amount of frothy, blood-tinged fluid. The trachea was normal. The peribronchial lymph nodes were small and gray. The right lung was similar to the left but without pleural tumor.

The spleen, pancreas, suprarenal glands, kidneys and urogenital tract were normal.

The gastro-intestinal tract was normal, except for marked congestion and petechial hemorrhages of the gastric mucosa.

The liver weighed 910 Gm. The surface was smooth and the cut surface normal in color and consistency. The gallbladder and ducts were normal.

There was a collection of flat, green nodules in the pelvis, firmly adherent to the sacrum.

The mediastinal, mesenteric, inguinal and retroperitoneal lymph nodes were slightly large and pink.

The marrow of the vertebrae, ribs and clavicles was brownish green.

*Microscopic Examination.*—Stains: The paraffin sections were stained with hematoxylin-eosin, hematoxylin-van Gieson and Giemsa's stains.

*Tumor Tissue:* The orbital tumors revealed a growth of closely packed, large oval, round and polyhedral mononuclear cells without tendency for orderly arrangement except in areas where they definitely followed thin-walled capillaries. There was a delicate network of stroma. Most of the cells were myeloblastic, with a thin, nongranular, uniformly staining cytoplasmic ring, more prominent on one side, and with a definite limiting membrane. Many cells contained slightly granular, neutrophil cytoplasm, while many others took the eosin stain and were clearly myelocytes. In the majority of the cells, the nucleus was prominent, larger than an erythrocyte, eccentrically placed, chiefly oval but often indented, occasionally bilobed or with two nuclei, vesicular, and contained one or more prominent nucleoli with a fine filamentous reticulum of chromatin and very rare mitotic figures. Adult neutrophil polymorphonuclear cells were rare. Every field contained eosin-staining cells varying from early myelocytes to adult leukocytes. Occasional basophil myelocytes were seen. The immature leukocytes were predominately positive to the peroxidase reaction. In the thin endothelial walls of the capillaries were occasional large oval and spindle-shaped cells, with prominent, large, oval nuclei that extended into the lumen or into the collection of myeloid cells that surrounded the vessels. Among the immature leukocytes were many early normoblasts, which took the basophil stain, and which were in all stages of hemoglobin content up to that of mature erythrocytes. There were many megaloblasts. Large fat cells were scattered through the sections, and several small nerves were seen.

Sections of the large dural tumor were similar to those of the described orbital masses, but with the eosinophil elements more pronounced. The neoplasm had almost replaced the entire dural tissue in areas and irregularly eroded the inner table of the skull, leaving spicules of bone with the spaces between and the marrow spaces of the diploe filled with immature myeloid cells as previously described.

*Brain:* The subarachnoid space over the left occipital lobe near the defect caused by the dural nodule was packed with large, immature myeloid cells with many eosinophil cells and a few erythroblastic cells. There was no invasion of the nervous tissue by the neoplasm, except for occasional small perivascular infiltrations.

The cerebellar meninges were rather markedly congested and contained occasional myelocytes in the spaces. There were many small areas of hemor-

rhage with moderate infiltrations of immature myeloid cells and a few normoblasts in the outer gray stratum of the cerebellar cortex.

No infiltrations were found in or about the optic nerves.

**Marrow Smears:** Giemsa-stained smears of rib marrow contained numerous large, immature leukocytes ranging from myeloblasts to granular myelocytes, chiefly neutrophil, but many taking the eosin stain and occasionally showing mitosis. A moderate number of adult eosinophil leukocytes, occasional neutrophil and rare basophil cells were present. Erythrocytes were relatively scarce; a few of these were megaloblasts; many were normoblasts. A few large phagocytic cells contained blood pigment and fragments of cells. Megakaryocytes were extremely rare and degenerated. Smears from sternal and vertebral marrow presented the same striking picture of a large number of early myeloid cells, many eosinophil cells and relatively few red elements.

**Bone:** Sections of decalcified rib presented enlarged marrow spaces, filled with closely packed early myeloid cells and a few adult leukocytes, with many cells taking the eosin stain. Erythrocytic cells were noticeably few and gathered in small islands with many normoblasts. The bony structure was very thin in areas. In one portion, the marrow cells were just beneath the periosteum with only narrow strands of fibrous tissue separating them from a large tumor adherent to the periosteum composed of cells similar to those found within the marrow spaces and in the orbital and dural tumors. Sections of the sternum were similar to those of the rib but with small islands of erythrocytic tissue more prominent.

**Liver:** The liver cells contained many fat droplets. Several medium-sized branches of the portal vein contained many myelocytic and myeloblastic cells among the red cells. A few early myeloid cells and occasional normoblasts were in the capillary sinusoids.

**Spleen:** There was marked dilatation of venous sinuses, which were filled with red cells, large mononuclear, myelocytic and myeloblastic cells with many eosinophil and basophil cells and rare normoblasts. In areas, the pulp cells were crowded into insignificance. The splenic nodules were small and in sharp contrast to the larger collections of immature leukocytes. Within a few of the nodules were moderate-sized areas of these cells.

**Lungs:** The pleura was thickened by a heavy infiltration of myeloid cells, eosinophil leukocytes and occasional normoblasts. A moderately large lymphatic vessel was packed with similar cells. The alveolar walls were thickened; the capillaries were congested and contained a few immature myeloid cells. Many of the alveolar spaces were filled with edema and fibrin containing large, granular, pigmented mononuclear cells, round cells, red cells and polymorphonuclear cells. Other spaces were atelectatic. Another section revealed practically all the alveolar spaces filled with edema, a few large, granular phagocytes, round cells and red cells, while others were packed with red cells. In patchy areas, a few alveoli were filled chiefly with early myeloid cells. There was a moderately large collection of myelocytic cells with a few eosinophils in the lymphoid tissue in strands of interlobular connective tissue.

**Heart:** The epicardium contained a moderate amount of fat with rather marked areas of hemorrhage and moderate-sized collections of myeloid cells with only a few eosinophil cells and rare normoblasts. There were many moderate-sized areas of hemorrhage in the myocardium, with several isolated infiltrations of the myeloid cells between the muscle fibers and about the vessels. The aorta was normal.

**Kidneys:** There were marked areas of congestion. The glomeruli were normal, with slight cloudy swelling of the tubular epithelium and a slight amount

of granular material in the tubules. In the interstitial tissue at the apex of a renal pyramid were moderate numbers of large myelocytic cells, a few eosinophil cells and many basophil cells. No other cellular infiltrations were found.

**Lymph Nodes:** The lymph nodes presented a rather marked diffuse lymphoid hyperplasia with the small, deeply staining lymphocytes in contrast with the large, lighter staining myeloblastic and myelocytic cells, which were in moderate numbers in the sinuses and through the more compact lymphoid areas. Eosinophil cells were plentiful. There were a few basophil cells and rare normoblasts. A few carbon-laden large mononuclear cells were found in the peribronchial nodes.

**Ovary:** There were many primary and occasional vesicular follicles in the ovarian cortex. In the fibrous connective tissue of the medulla toward the hilus was a diffuse infiltration of large myelocytic cells with a generous sprinkling of eosinophil mononuclears. There was one moderately large area containing numerous closely packed cells of this type with erythroblastic cells that closely resembled the orbital tumor.

**Uterus:** No infiltrations of the myometrium were seen, but the larger venules were packed with large myeloblastic and myelocytic cells with only occasional eosinophil cells.

**Other Organs:** The remaining organs were free from pathologic changes, and chloromatous infiltrations.

**Bacteriologic Smears:** Smears of the lungs contained many gram-positive lanceolate diplococci with a tendency to cling together in short chains. Rare single gram-positive cocci were in the smears from the vertebral marrow, while those from the sternal marrow and orbital tumors were negative.

#### COMMENT

The chloroma under discussion first appeared as a tumor attached to the periosteum of the orbit. It was olive-green and microscopically was composed of myeloid cells and primitive erythrocytic cells, while immature myeloid cells and erythrocytes were found in the patient's blood stream. The continued presence of immature myeloid cells in the blood after the removal of the only demonstrable tumor was confirmative evidence of the systemic nature of the disease and of the general involvement of the bone marrow that is typically found in chloroma. Because of this, postoperative irradiation was planned so as to give small, filtered doses of the x-rays to the entire body, in an effort to control the progress of the disease. While in Dock's reported series the duration of life was from five to nine months, Washburn<sup>6</sup> has on record a proved case of chloroma with multiple foci of bone destruction, mediastinal masses, intradural tumors and a leukemic blood picture that responded well to multiple small doses of the x-rays. Three and one-half years after operation for removal of the intradural chloroma, which was followed by irradiation of the whole body, the patient was living and apparently well. The defects of the bony skeleton and the mediastinal metastasis had disappeared, and the blood picture was normal. This one reported case was sufficient to recommend prolonged

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6. Washburn, A. H.: *Am. J. Dis. Child.* **39**:330, 1930.

small doses of the x-rays in the present instance, but they were without influence on the progress of the disease.

The progress of the disease was marked by rapid progressive anemia and thrombopenia, with symptomatic purpura. It is of interest that throughout the bone marrow and the several masses of abnormal tissue, megakaryocytes were extremely scarce. One might assume that in the rapid replacement of the normal marrow by the chloromatous tissue, the megakaryocytes giving rise to platelets were crowded out.

The presence of megaloblasts and normoblasts outside of the blood vessels, and forming part of the chloroma, is a finding that a study of the available literature on chloroma fails to duplicate. The inclusive term of chloro-erythroblastoma may well be used to describe this picture and to call attention to the erythroblastic potentialities in chloroma. Cases of multiple myeloma in which the typical cells were erythroblasts have been reported by Ribbert,<sup>7</sup> Froboese<sup>8</sup> and Schridde,<sup>9</sup> and in these cases the tumor has been given the name of erythroblastoma. Ewing<sup>10</sup> mentioned that Norris had encountered such a tumor, and referred to a specimen that he himself had studied which might fall within this group. In the instances described by Froboese and by Schridde, myeloid cells were present as well. Not only does the presence of both types of cells in the chloroma point to the close relationship of chloroma to multiple myeloma, much as the leukemic and aleukemic stages of leukemia are linked together, but it calls attention again to the interdependence of the myeloblastic and erythroblastic activities of the bone marrow, which is often lost sight of in the present rigid classification of diseases of the blood-forming organs. This is borne out again by the reported cases of polycythemia vera which in their terminal stages have shown the blood picture of leukemia.<sup>11</sup> The participation of both myeloblastic and active erythroblastic cells in an actively growing tumor, with the appearance of both types of immature cells in the blood stream, should be presumptive evidence for the monophyletic origin of the mammalian blood cells derived from bone marrow. In the study of the morphology of the individual cells of the tumor here reported, it was found impossible to identify a common parent stem cell giving rise to both erythroblasts and myeloblasts. The early myeloblasts were char-

7. Ribbert, Hugo: *Zentralbl. f. allg. Path. u. path. Anat.* **15**:22, 1904.

8. Froboese, C.: *Virchows Arch. f. path. Anat.* **222**:291, 1916.

9. Schridde, cited by Helly, K.: *Leukaemia*, in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histology*, Berlin, Julius Springer, 1927, vol. 1, sect. 2, p. 1061.

10. Ewing, James: *Neoplastic Diseases*, ed. 3, Philadelphia, W. B. Saunders, Company, 1928, p. 326.

11. Askanazy, M.: *Knochenmark*, in Henke and Lubarsch (footnote 9, vol. 1, sect. 2, p. 948). Weber, F., and Bade, O. B.: *Klin. Wchnschr.* **9**:2244, 1930.



acteristic, as were also the megaloblasts. Endothelial cells could be seen budding off from the wall of the vessel either into the lumen or to the outside to lie among the myeloid cells of the tumor. This budding off process has been described by Maximow<sup>12</sup> and has been frequently seen by Sabin<sup>13</sup> in the living chick embryo. During the budding off process, the cytoplasm and the nucleus take on the characteristics described under the heading Microscopic Examination, and reticulum cells were also encountered undergoing a similar transformation. No intermediate cells could be found that could be identified as the forerunners of the myeloblast or of the megaloblast. Since Sabin has shown that erythroblasts are the first blood cells to differentiate from the endothelium of the embryo, a tumor containing the forerunners of the erythrocyte may be looked on as being derived from a more primitive type of cell than those tumors composed purely of myeloid cells.

#### SUMMARY

A myeloid chloroma is reported in the formation of which both myeloblastic and erythroblastic types of cells participated, for which the descriptive term of chloro-erythroblastoma is suggested.

The close relationship of chloroma to multiple myeloma is indicated.

While the tumor here reported suggests the monophyletic origin of blood cells, no parent stem cell could be identified.

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12. Maximow, Alexander: *Arch. f. mik. Anat.* **63**:444, 1909.

13. Sabin, Florence R.: *Physiol. Rev.* **2**:38, 1922.

# THE PATHOGENESIS OF BRONCHIOLITIS OBLITERANS

OBSERVATIONS IN CASES OF BRIGHT'S DISEASE \*

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Obliterative bronchiolitis, described first by Lange (1901), is understood to be a secondary disease of the terminal bronchi following acute or subacute and, exceptionally perhaps, chronic inflammation of the bronchiolar walls. On the one hand, it occurs as a sequel of infectious pneumonia associated with marked involvement of the bronchial walls, as in measles (Hart), diphtheria (Colombino), influenza (Huebschmann; Opie, Blake, Small and Rivers) whooping cough (Lauche) and occasionally acute lobar pneumonia (Hart, Beitzke, Floyd, Lauche). On the other hand, it occurs in pneumonia caused by chemical irritants and associated with severe necroses; that is to say, after inhalation of ammonia or of sulphuric, hydrochloric or nitric acid (Fraenkel, Edens), as well as after inhalation of war gases (Winternitz, Groll, Adelheim). In both groups the most marked destruction of the terminal bronchi occurs.

Whereas in the first group the organization usually starts about fourteen days after the onset of the disease (Floyd), in the latter group it makes its appearance much earlier. Winternitz, in his experimental studies of war gas poisoning in dogs, observed it as early as five days after the onset. In our experiments with alcohol in rabbits, it was evident as early as three days. If one takes into consideration the fact that in the one group the condition follows slowly progressing bacterial inflammation and in the other group acute cauterization, the differences in time of onset are easily understood.

Besides these two groups of cases with known etiology there are reported in the literature isolated cases in which the etiology could not be determined (Lange, Galdi, Fraenkel, Vogel, Dunin-Karwicka).

In this study are presented several cases of obliterative bronchiolitis observed in Bright's disease that differ, at least in pathogenesis, from the cases of the groups mentioned. In these cases it has not been a matter of single organized plugs, which are certainly found more

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frequently than is generally believed (Floyd), but of an extensive process involving the whole lung or a major part of it. Only when the process is widespread throughout the lungs is it justifiable to designate the condition as obliterative bronchiolitis.

Descriptions of the clinical features of these cases and of the anatomic changes found in the kidneys have been published elsewhere (Van Slyke). Attention will be given here, therefore, chiefly to the pulmonary lesions.

#### PULMONARY OBSERVATIONS IN THREE CASES OF BRIGHT'S DISEASE

CASE 1.<sup>1</sup>—*History*.—M. K., a woman, aged 48, was admitted to the hospital two and a half months before she died. Uremic symptoms were present during the entire time that she was under observation, and she died in uremic coma. The clinical diagnosis was "arteriosclerotic Bright's disease. Latent-terminal."

On physical examination at the time of admission, the patient presented no pulmonary lesions. On June 6 (eight days before her death), she complained of tightness across the chest, for which there was no apparent cause. In the evening, difficulty in breathing developed. On the next day, the respiratory rate was increased, and the patient appeared to be very ill. There was marked dulness at the bases of both lungs, over which loud, moist râles could be heard. There was a little cough, with a very small amount of sputum streaked with fresh blood. The temperature was 100.2 F. On June 8, the temperature and the respiratory rate returned to normal. A roentgen examination showed heavy mottling of both lung fields, but there was no evidence of free fluid at either base. On June 10, the patient became comatose. Moist râles were present throughout both lungs, but especially in the left. The temperature again rose to 101.2 F. The roentgenogram showed more mottling than appeared two days before. Death occurred on June 14.

*Anatomic Diagnosis at Autopsy*.—At autopsy, the diagnosis was: primary contracted kidneys, malignant sclerosis of Fahr, cardiac hypertrophy, serous pleurisy, serous fibrinous pericarditis and cartilaginous perisplenitis.

*Postmortem Observations in Lungs*.—The right lung weighed 360, the left 330, Gm. The surfaces were apparently normal. The lobes were voluminous and firmer than normal and contained air. The cut surfaces had a mottled appearance. Those of the upper lobes were grayish red; those of the lower lobes, dark red. From the cut surfaces exuded a large amount of foamy fluid.

*Microscopic Examination*: Many of the alveoli of the lungs were filled with exudate; others contained air. In the upper lobes, the exudate consisted mainly of serous fluid, mixed with varying numbers of swollen mononuclear cells. Everywhere some erythrocytes and occasional polymorphonuclear leukocytes were found. Certain of the mononuclear cells contained several nuclei and were filled with fat, erythrocytes and coal pigment. In the lower lobes, the exudate contained many erythrocytes and varying numbers of mononuclear cells. The capillaries were dilated with blood. In their lumina increased numbers of polymorphonuclear leukocytes were present. The bronchioli were well preserved. The epithelial cells in places were desquamated. In the walls single polymorphonuclear leukocytes were

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1. The three cases discussed in this paper are cases 23, 38 and 53 in Dr. Van Slyke's monograph.

seen. The walls of the larger bronchi and blood vessels were edematous. Around the larger bronchi there were relatively many histiocytes filled with coal pigment.

Equally dispersed throughout all lobes, but in larger numbers only in the lower lobes, were small areas, some of which were round, others elongated, and still others leaf or trefoil-like in shape. These areas were situated chiefly in the bronchioli, alveolar ducts and alveoli. These areas represented sections of plugs consisting of erythrocytes mixed with a little fibrin, single polymorphonuclear leukocytes and mononuclear cells. Others (fig. 1) consisted of granular and partly fibrous sub-

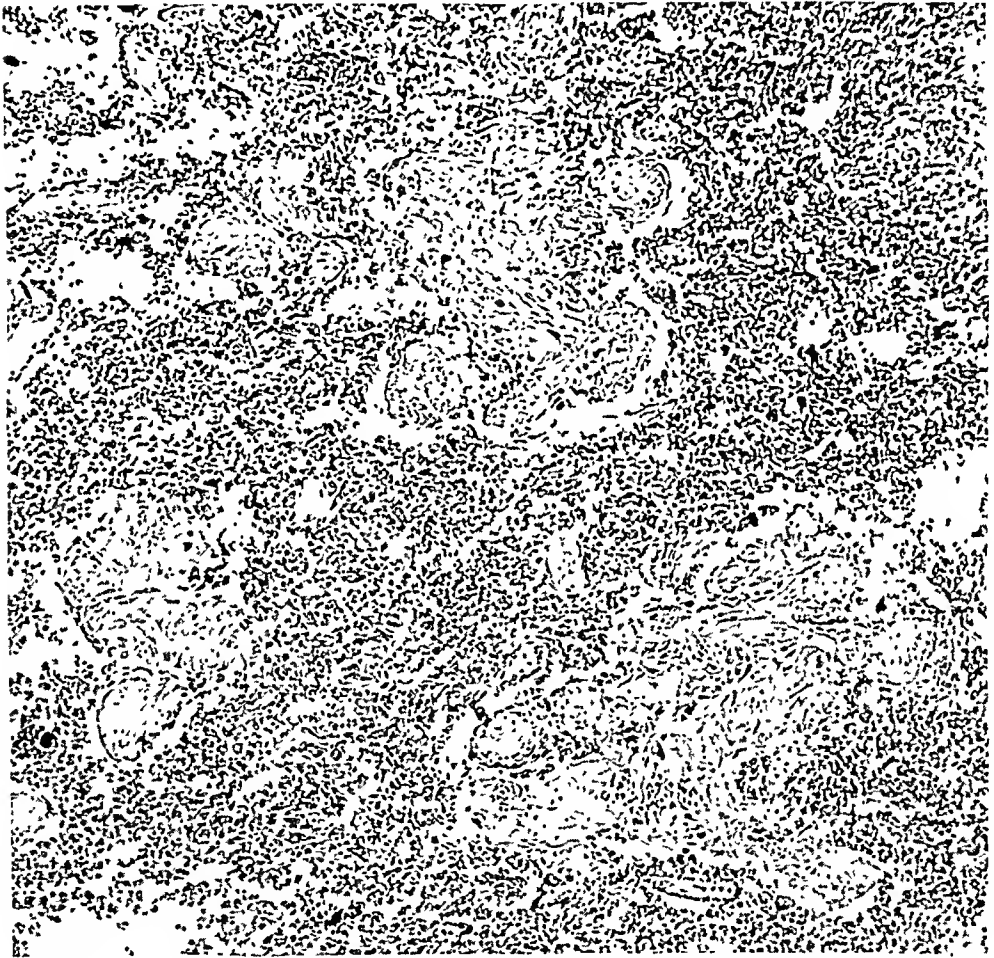


Fig. 1 (case 1).—Lung; iron-hematoxylin-eosin;  $\times 140$ : The alveoli are in part collapsed and contain many erythrocytes, varying numbers of mononuclear cells and single polymorphonuclear leukocytes. The capillaries are dilated with blood. Some acinous plugs consist of fibrinoid masses surrounded and penetrated by young fibroblasts.

stances, stained pink by hematoxylin-eosin, giving a positive fibrin reaction, and containing varying numbers of degenerated erythrocytes, mononuclear cells and single eosinophilic leukocytes, round cells and plasma cells. The areas were surrounded and penetrated by spindle-like cells with longish, pale nuclei. Distinct fibers were stained red by van Gieson's stain. No blood vessels could be found in these areas. The bronchioli filled by such plugs contained in places well preserved

epithelial cells, which, however, were not present on the plugs. Occasionally, connections between the plugs and the walls of the bronchioli could be observed. The walls of the bronchioli were well preserved. No cellular infiltrations or changes in the lymph vessels were seen. No bacteria were found.

CASE 2.—*History*.—E. S., a woman, aged 31, was admitted to the hospital several times before her last admission seventeen days before she died. Uremic symptoms had been present for a long time and persisted until the time of death. The clinical diagnosis was "hemorrhagic Bright's disease, terminal."

The patient had always been subject to sore throat. At the age of 24 she suffered from pleurisy, on account of which she spent two weeks in bed. Other respiratory disease had not been noticed by the patient, nor were abnormal pulmonary signs found on repeated examinations. About five weeks before the last admission she caught a cold, which developed into bilateral sinusitis. Since then she had been weak, and her health had become continuously worse. About one week before admission (from three to four weeks before her death), she became short of breath and complained of a dry cough. A sense of constriction about the chest was present, especially on the right side below the clavicle.

On admission, the breathing was rapid and somewhat embarrassed. Tactile fremitus was diminished at the right base, where there had been old adhesive pleurisy. The breath sounds were vesicular and tended to be harsh; they were diminished at the right base posteriorly. At the left base there were numerous moderately coarse, moist râles. The voice sounds were diminished over the dull area and exaggerated elsewhere. In a roentgenogram, taken sixteen days before death, rather fine mottling of the lung fields was seen, arranged in a radial fashion along the course of the bronchial tree and especially marked at the periphery of the lungs.

*Anatomic Diagnosis at Autopsy*.—At autopsy, the diagnosis was: secondary contracted kidneys, chronic glomerulonephritis, cardiac hypertrophy, fresh serous fibrinous pleurisy, old adhesions of the right lower lobe, fresh serous fibrinous pericarditis, fresh perisplenitis and perihepatitis.

*Postmortem Observations in Lungs*.—The right lung weighed 500, the left 390, Gm. Both lungs were voluminous and firmer than normal. Both contained air. The cut surfaces of the upper lobes were grayish red; those of the lower lobes, dark red. From the cut surface exuded a large amount of frothy fluid. The mucous membranes of the bronchi were reddish and covered by mucus.

*Microscopic Examination*: Many of the alveoli, especially those of the right lung, were filled with exudate, but others contained air and only a little exudate. The exudate consisted of serous fluid in which there were numerous swollen mononuclear cells. Erythrocytes were found everywhere, but especially in the lower lobes. There were also a few polymorphonuclear leukocytes and a little fibrin. Many mononuclear cells contained several nuclei as well as erythrocytes and fat. Others contained coal pigment. The capillaries were dilated with blood, in which there was obviously an increased number of polymorphonuclear leukocytes. The larger bronchi were in general well preserved. The walls of certain of them were infiltrated by varying numbers of polymorphonuclear leukocytes; a few leukocytes were also found within the lumina. The walls of many larger bronchi and of the arteries were edematous. Surrounding the small bronchi and bronchioli and also the smaller blood vessels were accumulations of histiocytes filled with coal pigment. The bronchial epithelium was frequently desquamated. In the walls of the small bronchi and bronchioli, as well as around the smaller blood vessels, were small round cell infiltrations and a few polymorphonuclear leukocytes.

Throughout the lungs there were small acinous areas situated in the bronchioli, alveolar ducts and alveoli, which consisted, on the one hand, of erythrocytes, fibrin, varying numbers of polymorphonuclear leukocytes, fibrin and mononuclear cells containing coal pigment and, on the other hand, of spindle-like cells. In many instances, these masses filled the lumina entirely (fig. 2). The cells contained longish, pale nuclei. There were many fibers, which were red when stained by van Gieson's method and blue when stained by Mallory's. The plugs contained no blood vessels. At their distal ends, one found occasionally a little fibrin, round cells and a few polymorphonuclear leukocytes. The acini filled by such plugs contained usually well preserved epithelial cells. Occasionally connections between the plugs and the walls of the bronchioli could be demonstrated. No bacteria were found.

CASE 3.—*History*.—F. S., a man, aged 44, was admitted to the hospital in a uremic condition six weeks before his death. The clinical diagnosis was "hemorrhagic Bright's disease. Acute-terminal. Acute arthritis."

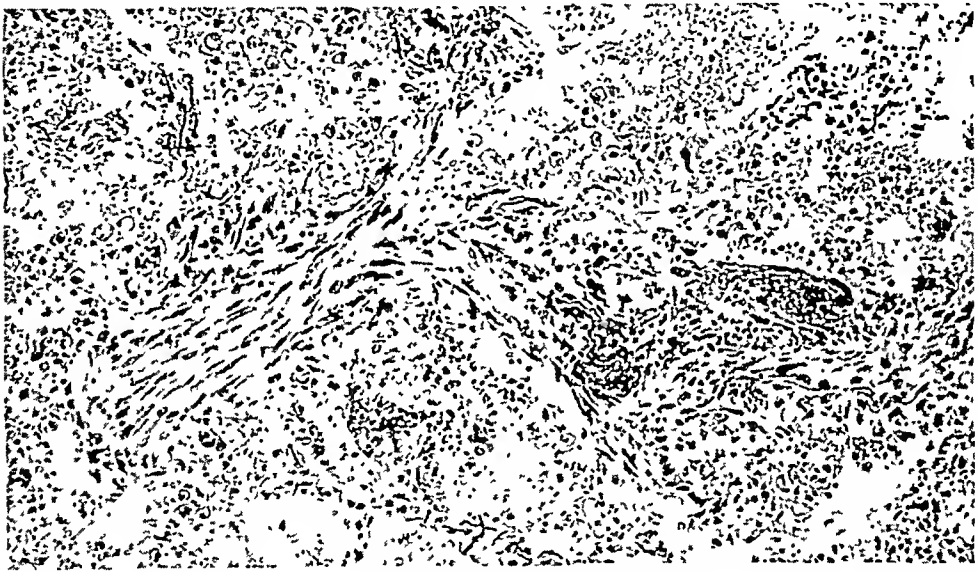


Fig. 2 (case 2).—Lung; methylene blue-eosin;  $\times 140$ : The alveoli contain serous fluid, mononuclear cells and single polymorphonuclear leukocytes. In the alveolar septums one sees fibrin. An acinous plug is to be noted that consists of older connective tissue, at the peripheral end of which fibrin and some leukocytes can be recognized.

Four months before admission, he suffered from a series of colds. Three months before admission, he contracted a severe cold, suffering from malaise, cough, expectoration and coryza. The cough improved, but he remained weak. Joint symptoms then appeared. On physical examination at the time of admission, no abnormal signs were found. A roentgenogram showed mottling of the lung fields in a radial fashion following the course of the bronchial tree. This appearance was especially marked at the hilus of the lungs. The apexes were relatively clear. Three days before the patient's death, the temperature, pulse rate and respiration rate all rose. There was dulness at the bases of both lungs posteriorly, and many coarse râles were heard.

*Anatomic Diagnosis at Autopsy*.—At autopsy, the diagnosis was subacute glomerulonephritis, extracapillary type of Fahr, aplasia of the right kidney, fresh

serous fibrinous pleurisy, old adhesions in the left pleural cavity, serous pericarditis, ascites, old adhesive perisplenitis and perihepatitis.

*Postmortem Observations in Lungs.*—The right lung weighed 990, the left 740, Gm. The surface of the left lung was covered by masses of fibrin. The lungs were voluminous and firmer than normal. In most places they contained air. The cut surface was dark and exuded a large amount of frothy fluid. The lower lobes especially contained firm nodes, which consisted of small yellowish areas surrounded by red zones. The trachea and bronchi were filled with a reddish, frothy fluid. Their mucous membranes were dark red.

*Microscopic Examination:* Although most of the alveoli were filled with exudate, others contained air. The exudate consisted of serous fluid, many swollen

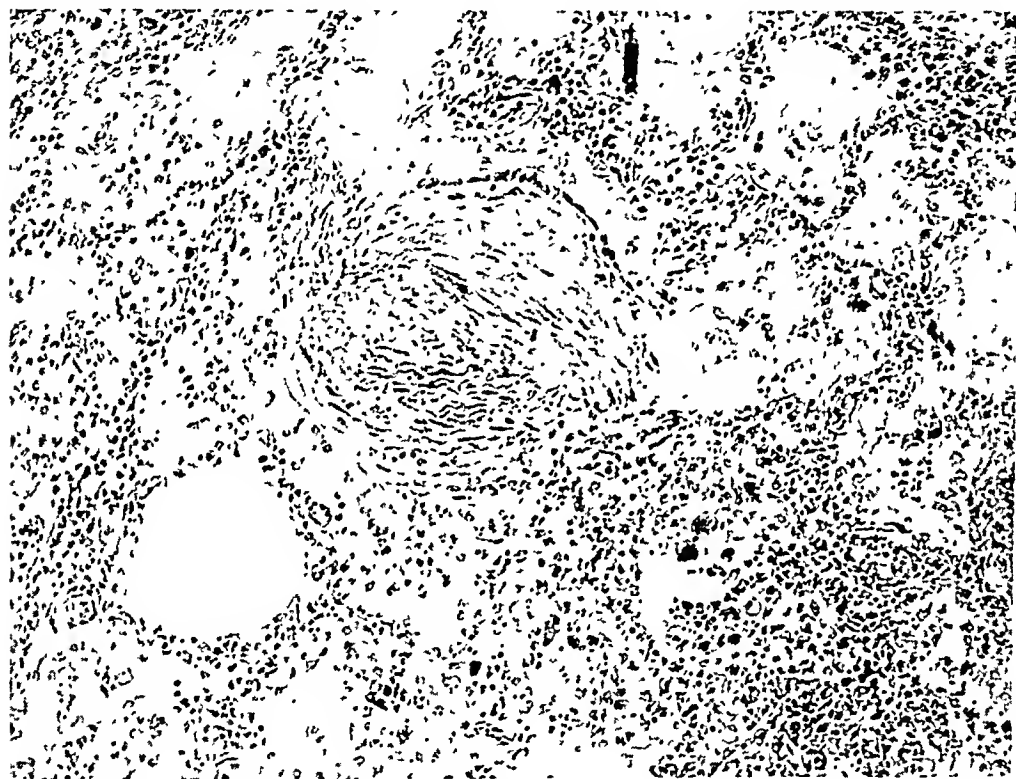


Fig. 3 (case 3).—Lung; van Gieson-elastic;  $\times 135$ : The alveoli are filled with serous fluid, mononuclear cells and a few polymorphonuclear leukocytes. The capillaries are rich in polymorphonuclear leukocytes. In the center of the field, one sees an old, edematous, organized plug, rich in collagenous fibers and poor in nuclei. The plug is covered by epithelial cells.

mononuclear cells and polymorphonuclear leukocytes. Some erythrocytes and a little fibrin appeared everywhere. Many mononuclear cells contained several nuclei and were frequently filled with erythrocytes, fat, carbon and a yellow pigment. The capillaries were dilated with blood and contained, as did the lymph channels, numerous polymorphonuclear leukocytes, which left them and penetrated the walls of the alveoli. The bronchi were usually filled with polymorphonuclear leukocytes and desquamated bronchial epithelial cells. Their walls, like those of the alveoli, were penetrated by numerous polymorphonuclear leukocytes. Around the larger bronchi accumulations of histiocytes filled with coal pigment were seen.

In all lobes larger bronchopneumonic areas were found, the alveoli and bronchi of which were filled with polymorphonuclear leukocytes, erythrocytes and fibrin. The centers of these areas were frequently necrotic and contained many gram-positive cocci. In the right upper lobe, a foreign body was seen in a bronchus in the center of a bronchopneumonic area.

Many areas, frequently edematous, were found in both lungs (figs. 3 and 4), which were partly roundish, partly bifurcated and partly stripe-like in shape. They consisted of collagenous connective tissue with thin, longish nuclei. That they were intrabronchial was clear from the arrangement of the elastic fibers and from the fact that they were sometimes surrounded by smooth muscles. Surrounding these areas (or plugs) distinct peribronchial tissue could be seen. They contained capillaries filled with blood. Between them and the bronchial walls occasional

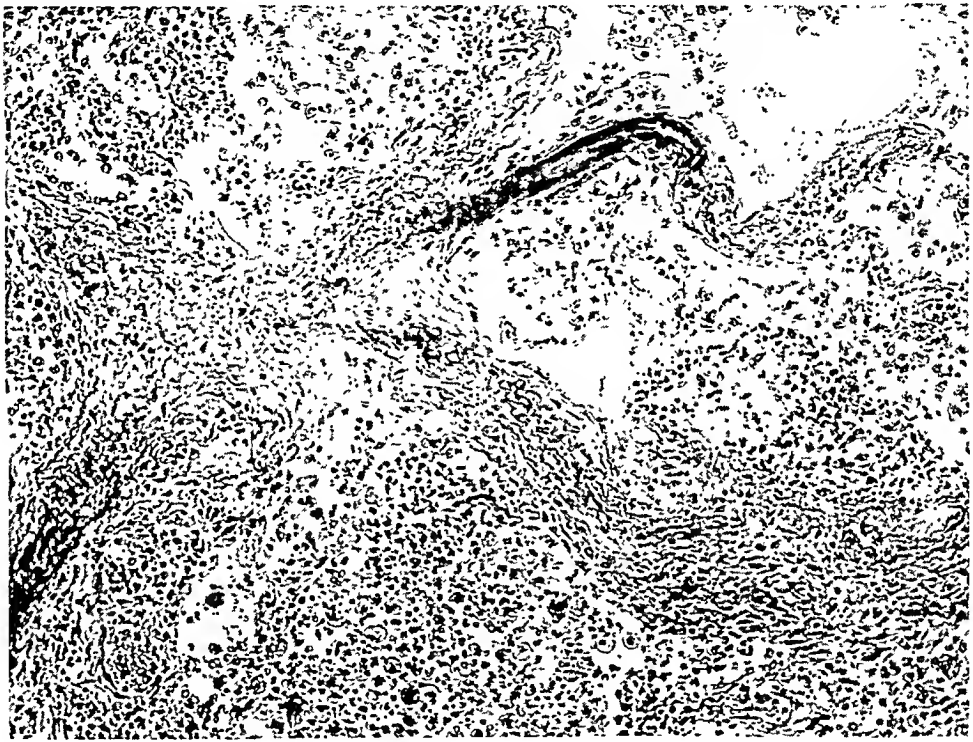


Fig. 4 (case 3).—Lung; van Gieson-elastica;  $\times 140$ : A treelike formation is seen, consisting of edematous connective tissue, rich in collagenous fibers and poor in nuclei. The surrounding alveoli are filled with mononuclear cells and some polymorphonuclear leukocytes. The capillaries are rich in polymorphonuclear leukocytes.

fissures appeared, the parietal walls as well as the surface of which were covered by epithelial cells (fig. 3).

These three patients with Bright's disease, dying in the state of uremia, exhibited extensive serositis, partly recent, partly quiescent. The lungs were edematous and congested. There was also, besides fresh pneumonic areas, older extensive obliterative bronchiolitis. In case 1, the first attack occurred eight days before death and was followed by a second one four days later. Histologically, there were besides fresh



acinous pneumonic areas, extensive plugs, already degenerated, into which fibroblasts had penetrated. In case 2, the first attack occurred from three to four weeks before death, whereas the terminal attack was unnoticed. Histologically, two separate and distinct attacks could, however, be identified. Besides fresh acinous pneumonic areas, older plugs were found that were nearly entirely replaced by connective tissue. In case 3, clinically, terminal confluent bronchopneumonia took place three days before death, its presence being demonstrated anatomically. There was, besides, extensive old obliterative bronchiolitis which, according to the clinical history and the histologic picture, must have developed about three months before death. In case 1, since neither clinical nor anatomic signs of descending infection were found, bronchiolitis must have begun in the terminal bronchi. In cases 2 and 3, descending bronchitis was demonstrable clinically as well as anatomically.

#### COMMENT

The demonstration of bronchiolitis obliterans in these cases has suggested an interest in two questions; first, that of its cause, and second, that of its pathogenesis. Why did resorption fail to occur, and why instead did the exudate become organized? The first question is easily answered in respect to the first two groups mentioned in the introduction, the lesion in the first group being due to an infection and that in the second to a noninfectious, chemical irritation. In the third group, which we shall now present, the cause is more difficult to explain. The occurrence of pneumonic lesions in patients with Bright's disease is well known. They were, in fact, absent only once in our cases. There can be little doubt that their frequency is due to a metabolic disturbance, characteristic of Bright's disease, resulting mainly from the tendency to edema and congestion. That this is so is recognized by the frequent occurrence of fibrinous effusions in the serous cavities of the body. In this mechanism, uremia probably is important. Aschoff thought that in this disease serositis is toxic and not bacterial in origin, though the pneumonias are caused apparently, at least in part, by infection. Infection is at all events responsible for the development of extensive confluent pneumonias, when there is distinctly a descending infection, as in case 2, as well as in the first attack in case 3. In case 1, however, the possibility of a toxic cause deserves consideration, just as in connection with general serositis, because of the absence both of descending infection and of cocci in the histologic sections. Unfortunately, no bacteriologic examination was undertaken in this case.

The answer to the second question is generally believed to be that the exudate which was for some reason not absorbed is responsible for the proliferation of connective tissue. Adelheim seems to be the only one to take the opposite view, namely, that proliferation is primary and

hinders the absorption of the exudate. He based this view on his observation that organization took place, in his experience, without the occurrence of exudation. This observation has not been confirmed in later examinations, nor is there a similar reference in the older literature. Lauche held that this view is, furthermore, contrary to experience with tissue cultures. In our cases, organization occurred only where coagulated exudate was present.

Why absorption does not take place in the first two groups is not yet definitely known. It is generally recognized that severe destruction of the walls of the bronchi is present (Hart and Mayer). C. and K. Hart and Beitzke, for example, expressed the belief that the destruction of lymph vessels results from destruction of the bronchial walls, and that the destruction of the lymph vessels is the cause of the failure of absorption, but Lauche maintained that the exudate is not transformed into a condition fit for absorption. For the latter view we found no evidence. The theory of Hart is supported, however, by the occurrence of this injury, as well as by the observation that in infectious bronchiolitis organization starts so much later than in that caused by chemical irritations. In the first group, absorption takes place early in the process and ceases only later when the bronchiolar walls are in an advanced stage of injury. In the second group, absorption ceases at once because of the immediate destruction of the bronchial walls.

In the third group that now is presented, destruction of the bronchial walls has not been found. It is true that in case 2 small infiltrations of round cells were found in the walls of the terminal bronchi and around the blood vessels, but they were certainly not sufficient to hinder absorption. Such lesions are frequently found in pulmonary and bronchial inflammations, and yet resolution takes place without the occurrence of organization. The most recent case (1) is of special interest, for in the bronchial walls only single polymorphonuclear leukocytes were found. Neither were signs of necrosis seen. It is necessary to look, therefore, for another cause of the failure of absorption. As has been mentioned, exudation is a common phenomenon in Bright's disease. There is, parallel with its occurrence, very little tendency to absorption, as is apparent in the frequency with which organization of the exudate in the cavities of the body is encountered. The answer to the second question appears, therefore, to be that the cause of the failure of absorption in Bright's disease is the disturbance of metabolism in the presence of uremia.

The problem concerning the origin of the connective tissue cells cannot be solved on the basis of the observations now presented. C. and K. Hart and probably most authors (Lauche) look on the terminal bronchi as the starting points of the organizing process. Huebschmann and Groll believed, however, that proliferation takes its origin in the

connective tissue ring at the base of the alveoli and from the walls of the alveoli proper. Since the alveolar walls contain relatively few fibroblasts (Lang, 1925, 1926), new-formed fibroblasts must be regarded as taking (according to Huebschmann and Groll) their origin in alveolar epithelium, as Floyd in fact believed, or in septal cells or in undifferentiated embryonal connective tissue cells. Although this possibility must be entertained (Maximow), new collagenous connective tissue is formed in the lung nearly exclusively from fibroblasts in the peribronchial and perivascular tissue, as Lang (1925, 1926) showed in cultures of pulmonary tissue. The present observations are, as those of most authors, in accord with this explanation. In many sections it seemed clear that the connective tissue originated in the walls of the bronchioli, whereas bridges connecting the plugs with the alveolar walls were usually absent.

#### SUMMARY

In the postmortem examination of certain cases of Bright's disease in which uremia was present at the time of death, fresh pneumonic areas were observed besides extensive lesions of preexisting obliterative bronchiolitis. Cases of obliterative bronchiolitis described hitherto are divisible in two groups depending on their etiology and pathogenesis. The severe destruction of the bronchial walls seen in them was missing in the third group now first described. Absorption of the exudate failed in this group because of the disturbance of metabolism which often occurs in the uremia of Bright's disease, and which manifests itself by increased tendency to exudation and decreased tendency to absorption. In two cases, at least, the occurrence of bronchiolitis was caused by bacterial infection. Whether it can arise as the result of intoxication alone, as does general serositis, could not be decided.

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# THE ENCEPHALITIS OF DOGS WITH ECK FISTULA FED ON MEAT\*

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AND

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Hahn, Massen, Nencki and Pawlow<sup>1</sup> in 1893 described the nervous symptoms that occurred in dogs with Eck fistula fed on meat. These symptoms consisted of excitement, ataxia, amaurosis and finally coma. According to the Russian school, this condition does not develop in all dogs with Eck fistula fed on meat, and if it develops, recovery often follows. These workers regarded the condition as poisoning due to carbamic acid brought about by the direct introduction of the blood from the portal vein into the circulation, on exclusion of the liver.

Bielka von Karltru was not able to reproduce the symptoms mentioned. Filippi found that not all the dogs with Eck fistula presented these symptoms while fed on meat, and that this intoxication also occurs with a mixed diet. Of eighteen dogs with Eck fistula fed on meat by Rothberger and Winterberg,<sup>2</sup> just seven became sick and only three severely. This proves that some dogs with Eck fistula are susceptible to this disease on a one-sided diet of meat, and that others do not react to meat feeding. No connection could be detected between the degree of meat feeding, the size of the Eck fistula or the development of the collateral circulation and the symptoms. The administration of ammonium salts to dogs with Eck fistula did not induce poisoning. An accurate analysis of symptoms indicated differences between the symptoms of dogs with Eck fistula and those due to carbamic acid poisoning.

Matthews and Miller<sup>3</sup> made Eck fistula in thirty-five dogs. When these dogs were given meat, toxic symptoms developed in only three. Magnus Alsleben stated that the one-sided nutrition with meat facilitates the development of toxic symptoms, but that these are in fact due to the inadequate function of the liver. Products of intestinal diges-

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\* From the Pathological Anatomical Institute of the Hungarian Royal Francis Joseph University; director, Prof. Joseph Baló.

1. Hahn; Massen; Nencki, and Pawlow: *Arch. f. exper. Path. u. Pharmakol.* **32**:161, 1893.

2. Rothberger and Winterberg: *Ztschr. f. exper. Path. u. Therap.* **1**:312, 1905.

3. Matthews and Miller: *J. Biol. Chem.* **15**:87, 1913.

tion that are either abnormal or increased in quantity induce disease if they reach the circulation, evading the liver filter.

These were the ideas regarding the etiology of the encephalitis of dogs with Eck fistula at the time of the publications of Economo on the epidemic encephalitis in 1918, which have given new trends to research. Fuchs<sup>4</sup> announced the disease of one Eck fistula-bearing dog as encephalitis, based on clinical findings. Pollak<sup>5</sup> corroborated this diagnosis in finding histologically diffuse encephalomyelitis. His dog died sixteen days after the production of Eck fistula and five days after the beginning of meat feeding. Fuchs succeeded, with the careful administration of guanidine hydrochloride, in producing in cats conditions corresponding clinically and histologically to the encephalitis. The symptoms of Eck fistula-bearing dogs and cats fed on guanidine were similar, namely: fascicular, choreic and clonic convulsions, drowsiness, furor, paralysis and finally coma. Fuchs believed that as a result of one-sided meat feeding methyl-guanidine and similar substances that are strongly toxic reach the circulation, evading the liver. According to him, the encephalitis of dogs with Eck fistula is guanidine poisoning.

Of the Eck fistula-bearing dogs of Kirschbaum,<sup>6</sup> three died of an intercurrent disease. In the brain of the fourth, killed ten days after continuous meat feeding, he found, in the absence of any abnormal clinical symptoms, an inflammatory condition. He regarded the explanation offered by Fuchs as unsatisfactory. According to Kirschbaum, the Eck fistula causes disturbances in metabolism that make possible the invasion of a virus into the nervous system. Silberstein and his collaborators<sup>7</sup> succeeded in producing with an average of five days of meat feeding in dogs encephalitis resembling human epidemic encephalitis. The encephalitis was transmissible to dogs and rabbits by subdural and corneal inoculation of a filtrate of the emulsified brain. These workers proved the identity of the virus from their Eck fistula-bearing dogs with that of human encephalitis by neutralizing the Eck fistula virus with human encephalitic antiserum, and the virus of human encephalitis with an antiserum against Eck fistula virus. These authors explained the finding that not all the dogs with Eck fistula react with encephalitis to the feeding of meat, stating that in the nasopharyngeal cavities in a certain percentage of normal dogs a virus can be detected that is supposed to be identical with the virus of human epidemic encephalitis.

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4. Fuchs: *Wien. med. Wchnschr.* **71**:710, 1921; *Arch. f. exper. Path. u. Pharmacol.* **97**:79, 1923.

5. Pollak: *Arb. a. d. neurol. Inst. a. d. Wien. Univ.* **23**:2, 1922.

6. Kirschbaum: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **88**:487, 1924.

7. Silberstein: *Wien. klin. Wchnschr.* **37**:30, 1924. Orel and Silberstein: *Ztschr. f. d. ges. exper. Med.* **44**:280, 1925. Hoff and Silberstein: *ibid.*, p. 268.

After the Eck fistula has been made in dogs, and after the administration of a meat diet, those dogs contract the encephalitis that bear in their nasopharyngeal cavities the supposed encephalitic virus. The exclusion or the abnormal function of the liver, i.e., disturbances of metabolism, bring about the invasion of an ubiquitous saprophytic virus, which in this case causes a fatal infection.

Fischler<sup>8</sup> found that the meat intoxication of dogs with Eck fistula can end fatally with normal histologic observations in the brain. Symptoms of meat intoxication are ataxia, amaurosis and hypesthesia. At the beginning, poverty of movements and catalepsy can be observed. György and Kleinschmidt,<sup>9</sup> in feeding dogs with a meat diet free from fat and bone, produced an intoxication that was followed by alkalosis and an increase of reserve alkali, blood sugar and blood lactic acid. These workers inoculated into guinea-pigs, rabbits and dogs brain from eight dogs that showed typical meat intoxication, but the inoculated animals did not contract encephalitis. Kleinschmidt<sup>10</sup> did not believe that there is any identity of the meat intoxication of Eck fistula-bearing dogs and epidemic encephalitis.

#### EXPERIMENTS

*Clinical Observations.*—Eck fistula was produced in eighteen dogs. Of these, ten died during the operation or shortly afterward. To eight dogs, from seven to twenty-five days after their complete recovery, a horse meat diet was given. The dogs reacted to the one-sided meat diet differently. One dog (11), which during a period of thirty days had not eaten well and had taken little meat, was not affected. Another dog (9), to which the meat had been given the earliest, seven days after the operation, showed on the fifth day of meat feeding slight nervous symptoms, such as ataxia, rotary steps, feebleness and salivation. These symptoms disappeared on the next day and did not appear again. Six dogs became sick, the first on the second day and the last on the twentieth day of meat feeding. The symptoms were severe in four dogs and ended fatally. Two dogs were killed when they presented severe nervous symptoms. Certain dogs showed many similarities in the clinical course of the disease; yet the onset of symptoms and their quality varied considerably.

Two dogs (22 and 10) became ill on the second and twentieth days, respectively. Their disease was severe and in three days ended fatally. In one dog (22), the symptoms progressed gradually, for in this dog, refractory and ataxic at the beginning, marked hypesthesia and amaurosis developed and on the third day tonic-clonic convulsions. These propagated from the extremities to the chewing musculature, and opisthotonos developed. The animal died in coma, lasting a few hours. In another dog (10), severe tonic-clonic convulsions developed, lasting only a few hours. Later on a profound coma followed, and the animal died in three days. In these two cases, the clinical diagnosis of encephalitis was with the greatest probability established.

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8. Fischler: *Physiologie und Pathologie der Leber*, Berlin, Julius Springer, 1926.

9. György and Kleinschmidt: *Ztschr. f. d. ges. exper. Med.* **54**:1, 1927.

10. Kleinschmidt: *Ztschr. f. d. ges. exper. Med.* **54**:20, 1927.

In three dogs (3, 20 and 23), the disease, though presenting as a whole similar symptoms, showed different courses. The severe nervous symptoms appeared in certain attacks. They disappeared in a few days and after an intermission of from eight to fifteen days reappeared. Dog 23 survived two and dog 20 three attacks, whereas dog 3 was killed during the third severe attack. All three dogs became ill at the beginning of the meat feeding, two on the fourth and one on the fifth day. Disinterestedness, poverty of movements, uncertain walk, hypotonia, hypesthesia and amaurosis developed. Sometimes they hit the wall with their heads and then remained in a bizarre position (catalepsy). To this constant symptom complex, in some cases, choreiform or clonic movements of moderate intensity or attacks of convulsions were joined (dogs 3 and 23). This condition lasted for from one to three days and was accompanied by a loss of appetite and fasting, which was followed by recovery. The dogs having recovered and having taken the meat again, after a period of from seven to fifteen days became ill again. Dogs 20 and 23 showed in the final stage of the disease certain differences. Whereas the former presented apathy, hyperesthesia, exaggerated reflexes, hypotonia, some fibrillary twitchings and salivation and finally died after a soporous condition lasting two days, in the latter, even on the last day of life, great excitement, compulsory movements, circus movements, rotary movements to the right around its axis were observed, with death following while the animal was in deep coma. In these three dogs, the periodic undulation of symptoms pointed to a toxic condition depending on meat feeding, but the diagnosis of encephalitis could not be denied.

Encephalitis was taken into consideration in the case of dog 7. This animal became ill on the fourteenth day of meat feeding, with tonic-clonic convulsions of great intensity. It was killed six hours after the onset of convulsions.

The nonprotein nitrogen in the diseased dogs was determined in several instances and found to be very high. In dog 10, before death, it was found to be as high as 145 mg. per hundred cubic centimeters.

*Postmortem Observations.*—The fistula between the portal vein and the vena cava was always found to be patent (from 16 to 10 to 5 mm. in length). The ligature of the portal vein made as a routine above the pancreaticoduodenal vein held well. The urinary bladder in all six dogs was distended, sometimes reaching above the umbilicus (1, 10 and 20). Hemorrhagic erosions and ulcers arising from hemorrhages were found in the stomach in two dogs (20 and 22) and gastric hemorrhages in one (23). Pancreatic fat necrosis occurred in one dog (20). Usually the brain was somewhat edematous. Other than these, the postmortem observations were negative; only the liver presented in all cases marked fatty degeneration.

*Histologic Observations in the Brain.*—The brains of all the experimental dogs were subjected to histologic examination. The brains from the dogs that died during or shortly after operation served as controls. The brains of six dogs that became ill after meat feeding were examined in detail. In making the histologic examination, we wanted to find out whether alterations corresponding to an inflammatory process, i.e., encephalitis, are present in the brains of dogs with Eck fistula whether fed on meat or not. Of six dogs, three (3, 7 and 23) showed no inflammatory alterations in the brain. Whereas the brains of dogs 3 and 23 did not contain histologic changes, that of dog 7 revealed the marked congestion of the meninges corresponding to the early stage of encephalitis. In the brains of three other dogs (10, 20 and 22) without any doubt inflammatory alterations were found, although these varied somewhat in nature. The process was localized to the corpus striatum.



In dog 10, the blood vessels of the meninges were congested. In the cerebral cortex and also throughout the central nervous system hyperemia and some capillary hemorrhages occurred. Alterations were found in the head of the caudate nucleus just below the ependyma. The ependyma was thickened and hyperemic, and its cells were increased in number. In the striated bodies beneath the ependyma, the precapillaries were surrounded by cells (fig. 1). The cells were partly round, uni-nucleated, the majority elongated, spindle-shaped, corresponding to lymphocytes and adventitial cells (fig. 2). The cells occupying the perivascular lymphatic spaces were often arranged in three or four layers, but sometimes they were only in one row. The process was limited to the head of the caudate nucleus.

The brain of dog 20 showed diffuse hyperemia. There were marked alterations in the head of the caudate nucleus below the ependyma of the lateral ventricles. Here the capillaries and the precapillaries were surrounded by cells that followed

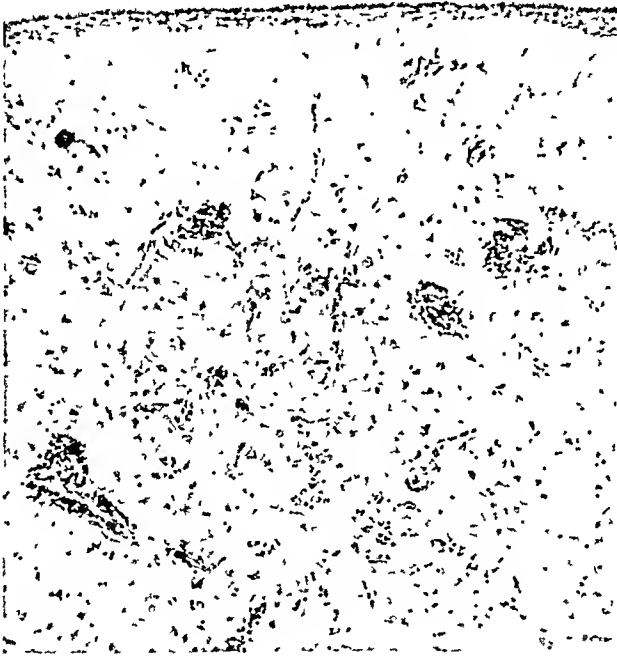


Fig. 1.—Perivascular cell accumulations below the ependyma covering the head of the caudate nucleus (seen with the low power lens in dog 10).

them in a mantle-shaped manner. The perivascular infiltration was caused by the accumulation of mononuclear cells, such as lymphocytes and plasma cells (fig. 3). In some places oligodendroglia and microglia cells were also increased in number, but these presented degenerative alterations. The endothelial cells of capillaries were very much swollen, filling the lumens almost entirely. In other parts of the brain no pathologic alterations were found.

In this case, as well as in the former one, the pathologic manifestations of encephalitis were found. The process was limited to the head of the striated bodies; thus we had to deal with a toxin that had an elective affinity to the striated bodies.

The brain of dog 22 differed somewhat from the brains of dogs 10 and 20. The leptomeninges, as well as all parts of the brain, were congested. In the larger blood vessels, intravascular accumulation of leukocytes was observed. In the leptomeninges, a diffuse increase in the number of cells was noted, without perivascular

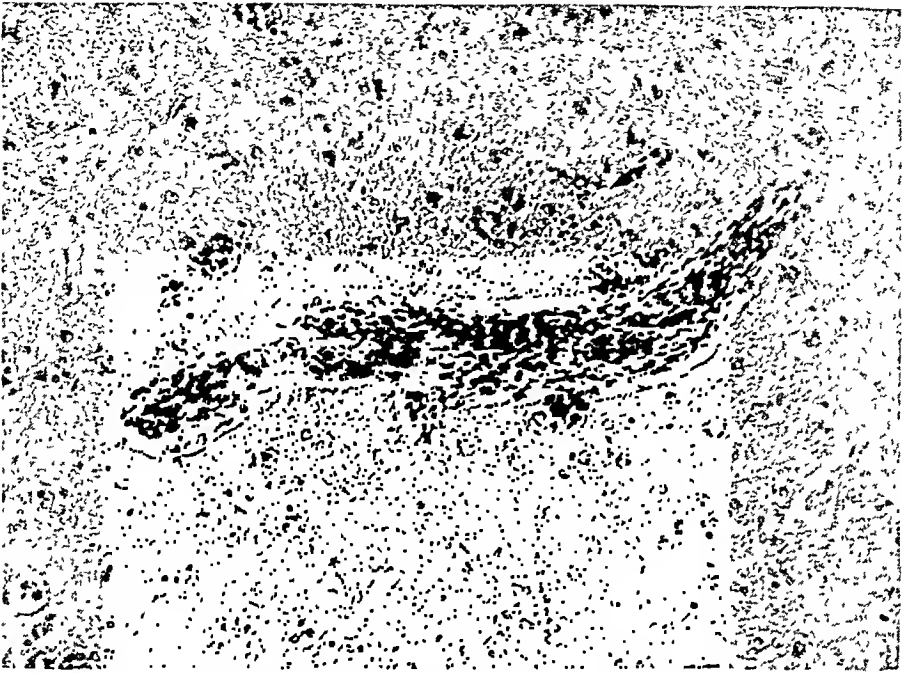


Fig. 2.—A perivascular cell accumulation composed mostly of adventitial cells (seen with the high power lens in dog 10).

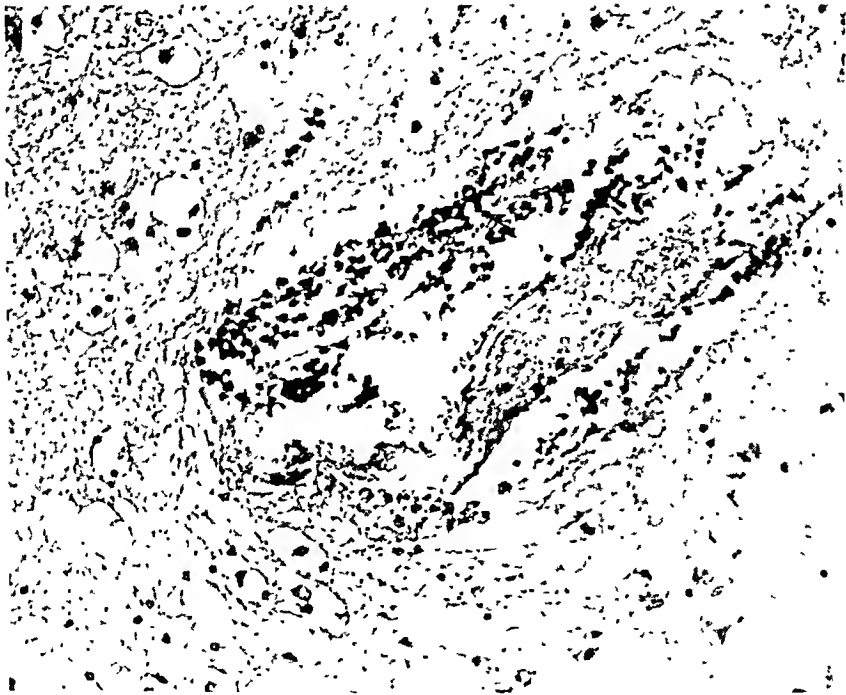


Fig. 3.—A perivascular round cell infiltration in the head of the caudate nucleus (seen with the high power lens in dog 20).

accumulation. At most, cells possessing very little cytoplasm with deeply stained nuclei and large cells of light protoplasm were found. Monocytes, plasma cells and macrophages were also increased in number. In some places, these cells entered the cerebral cortex from the meninges. In the cerebral cortex, as well as in other parts of the brain, besides marked congestion, slight perivascular infiltration occurred. The infiltrate was formed in some places by only a few mononuclear cells. The histologic diagnosis was disseminated meningo-encephalitis.

*The Question of Transmissibility.*—In three of six dogs that became ill in our experiments, we found histologic alterations corresponding to encephalitis. This could be interpreted in two ways. Either the meat poisoning itself caused the encephalitis, or the meat poisoning and the exclusion of the liver activated a virus that brought about encephalitis. The brain of three of our encephalitic dogs was inoculated intracerebrally into dogs and rabbits. With the fresh brain material of dog 10 we inoculated one dog. With the emulsion of the brain of the same dog kept seventy-two days in 50 per cent glycerin we inoculated two dogs and two rabbits. Intracerebral, subdural and intraspinal inoculations into dogs and rabbits were carried out with the brain material of dogs 20 and 22, which had been kept in 50 per cent glycerin for forty-four and twenty days, respectively. The inoculated dogs and rabbits did not contract encephalitis in a single case. The dogs died as a rule in from three to four weeks after inoculation, but as we reported previously, the cause of death was gastric hemorrhage due to erosions and ulcers following lesions of the brain. No histologic alterations could be detected in the brains of the inoculated dogs. Cell inclusions were also absent.

#### SUMMARY

Six of eight dogs with Eck fistula, fed on a one-sided diet of meat, became ill, presenting symptoms of encephalitis. We were unable to transmit the encephalitis to dogs; that is, the intracerebral inoculation of brain material led to fatal gastric hemorrhages arising from erosions and ulcers caused by minimal lesions of the brain during intracerebral inoculation. No histologic evidence of encephalitis was found in these dogs or in the rabbits inoculated. On the basis of these investigations, there is no evidence that the encephalitis of dogs with Eck fistula is caused by a living micro-organism or by a virus. We regard the encephalitis occurring with the one-sided meat diet and intoxication as due to the exclusion of the liver. The intoxication may follow a periodic course. The intoxicated dog does not eat; following fasting, the toxic symptoms disappear, but they reappear after the dog begins to eat meat again. An intoxication of mild degree does not necessarily bring about histologic alterations in the brain. Such alterations usually follow the attacks of encephalitis appearing at intervals. Fatal intoxication causes alterations in the brain corresponding to nonsuppurative encephalitis, which are localized in the caudate nucleus. The localization of the encephalitis of dogs with Eck fistula in the striated bodies again proves the relationship of abnormal liver function to the brain. The finding first pointed out in these experiments that the encephalitis of dogs with Eck

fistula is localized electively in the striated bodies brings a new prospect for the experimental study of the etiology of Wilson's disease and pseudosclerosis.

#### CONCLUSIONS

Six of the eight dogs with Eck fistula that were kept on a meat diet grew ill and showed symptoms of encephalitis. In the brains of three dogs, histologic alterations corresponding to nonsuppurative encephalitis were found.

The encephalitis of dogs with Eck fistula has a firm histologic foundation. It is localized in the striated body. This finding proves that the noxious agent has an elective affinity to the striated body.

The Eck fistula, the meat feeding and the metabolic disorders following them bring about the encephalitis. There is no proof that a virus that is in a latent condition in the body of a dog can penetrate into the nervous system and cause encephalitis following Eck fistula and intoxication due to meat feeding.

# HEMOCHROMATOSIS AND PRIMARY CARCINOMA OF THE LIVER

REPORT OF A CASE, WITH REVIEW OF THE LITERATURE  
AND DISCUSSION OF THE PATHOGENESIS \*

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The concepts of hemochromatosis are numerous and divergent. An association of hemochromatosis with carcinoma of the liver is rare, although it is believed that this is more common than carcinoma of the liver following cirrhosis without hemochromatosis (Milles, Althausen and Kerr). To the present time, fourteen cases of hemochromatosis associated with primary carcinoma of the liver have been reported (Löhlein, Milles, Althausen and Kerr, Rindfleisch, Donaldson, Stewart, Achard and Leblanc, Orr and Hibbs). Critchlow reported a case of hemochromatosis associated with primary carcinoma of the pancreas. Of the one hundred and sixty-five cases of hemochromatosis that have thus far been reported, 9 per cent were associated with primary carcinoma of the liver.

As all the cases of hemochromatosis presented cirrhosis of the liver, a comparison of the incidence of carcinoma of the liver following cirrhosis is of interest. In 2,091 consecutive autopsies at the Cook County Hospital in the years from 1929 to 1931, there were encountered sixty-eight cases of cirrhosis of the liver, or 3.2 per cent. There were twelve cases of primary carcinoma of the liver in this group, of which seven were associated with cirrhosis; so that 9.3 per cent of the cases of cirrhosis of the liver without hemochromatosis were associated with carcinoma.

In a comparison of the statistical results, the incidence of carcinoma of the liver following cirrhosis plus hemochromatosis is not greater than the incidence following cirrhosis alone. That cirrhosis of the liver predisposes to carcinoma is universally accepted (Counseller and McIndoe, Goldzieher and von Bokay, Eggel, Oertel, Goodpasture, Jaffé, Rolleston).

The case reported here is one of hemochromatosis in association with primary carcinoma of the liver. The histologic picture suggests the pathogenesis of hemochromatosis.

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\* From the Department of Pathology, Cook County Hospital; Dr. Richard H. Jaffé, director.

## REPORT OF CASE

*History.*—It was difficult to obtain a history from the patient, as he was deaf, so that each question had to be put to him in writing. He was a white man, 69 years of age, who in the last year had not been feeling well. He first noted swelling of the ankles, and then of the abdomen. The swelling of the latter was associated with pain and shortness of breath. He consulted the doctor, who noted an enlarged liver that was nodular. Gradually his abdomen became filled with fluid so that paracentesis was necessary, and a large amount of blood-tinted fluid was removed. Carcinoma of the liver was diagnosed and the patient was hospitalized.

On admission to the hospital, the patient complained of a dull aching in the umbilical region which was almost continuous, unless he was reclining, and of griping pains in the right upper quadrant that came on at one-half to one hour intervals and lasted for short periods. His appetite was fair, but he had lost a little weight.

*Physical Examination.*—The patient was emaciated and icteric, and appeared subacutely ill. There were two sebaceous cysts in the scalp. He was blind in the right eye because of a dense corneal opacity; the vision of the left eye was fair. He was completely deaf.

The chest was emphysematous, and there were a few moist râles in both bases. The heart was normal, but the radial vessels were markedly sclerotic.

The abdomen was distended, its superficial veins prominent. There was a shifting dullness. A serosanguineous discharge from the wound of the infra-umbilical paracentesis was noted. The liver was firm and nodular and extended to the level of the umbilicus. There was a pitting edema of the lower extremities.

The icterus index was 13. The urine had a small amount of albumin. The Kahn reaction of the blood was negative.

The clinical diagnosis was carcinoma of the liver, either primary or secondary to carcinoma of the gallbladder or of the stomach, with carcinomatous metastases in the peritoneum to account for the bloody ascites.

During his short stay in the hospital the patient complained of continual pain in the abdomen, and on the day of his death suddenly became cold and clammy, with a weak, thready, rapid pulse. Restlessness was marked, and the patient died with definite signs of air hunger.

*Postmortem Examination* (Dr. R. H. Jaffé).—The weight of the body was 125 pounds (56.7 Kg.); the length, 160 cm. It was that of a poorly nourished white man, aged 69. The skin was light dirty-yellow gray. The sclerae were slightly icteric.

The abdomen was distended to two fingerbreadths above the level of the chest. There was marked pitting edema of the lower extremities. The abdominal cavity contained 5,000 cc. of a thin, blood-tinted fluid. The liver was 11 cm. below the xiphoid process and 6 cm. below the right costal arch.

The pleural cavities were free from fluid or adhesions. The heart weighed 290 Gm. The left ventricle measured 16 mm. and the right ventricle 3 mm. The myocardium was pale, brownish-gray and friable; the chambers were dilated. In the posterior portions of the left lung there were dark areas of consolidation.

The spleen weighed 415 Gm. and was soft. The capsule was light gray, with numerous yellowish-white, firm plaques measuring up to 8 mm in diameter. The pulp in the periphery was deep purple, while in the central portions it was lighter—a purplish gray; the trabeculae were prominent.

The liver weighed 2,400 Gm. and measured 30 by 20 by 13 cm. The surface was deep brown, with numerous pinhead-sized, lighter gray-brown nodules and larger depressions, especially near the lower margin and on the inferior aspects of both lobes. In addition to these fine nodules, the left lobe contained many larger and softer ones, which were light purplish-pink and varied from 1 to 20 mm. in diameter. The larger nodes were raised as high as 16 mm. Arising from the lower third of the falciform ligament, there was a fist-sized, soft, roughly spherical tumor, which extended into the parenchyma of the liver. This node, to which the transverse colon was loosely attached, measured 10 cm. in vertical, 20 cm. in transverse, and 8.5 cm. in anteroposterior, diameter. The liver was of firm consistency, and the sectioned surface was deep brown, with a branched network of grayish-white lines, radiating from the portobiliary septums, that divided the liver into small islands of tissue, averaging 2 mm. in diameter. A coronal section of the falciform ligament revealed the lowermost portion of the liver to be substituted by a moderately firm mass, 6.5 cm. in anteroposterior, and 4.5 cm. in vertical, diameter. The mass was light pinkish gray, studded with light yellowish gray and pinkish red, passing into the softer mass of the falciform ligament. The portal vein and its main branches were occluded by soft, friable, light yellowish-gray tissue. The smaller nodules of the left lobe on being sectioned were light yellowish gray. The larger node in the falciform ligament was covered by soft, dark red blood clots, which protruded from an irregular tear on the left side. The soft tumor in the falciform ligament was light gray and centrally liquefied.

The gallbladder was distended and filled with a thin, light green bile. The mucosa was light green and smooth. The extrahepatic bile ducts were patent.

The pancreas weighed 185 Gm. and measured 24 by 4 by 3 cm. It was firm and, on being sectioned, showed prominent light brown lobules. The lobules were separated by an increased amount of fat.

The mucosa of the cecum was dark purplish red. The mucosa of the ileum was pale pink, with small bright red areas, and was covered by an increased amount of mucus. The entire wall was thickened and edematous. The descending colon presented numerous outpouchings of the wall, which were as much as 1 cm. deep and 1.5 cm. in diameter and lined by a smooth mucosa.

The peripancreatic lymph glands were soft, measured as an average 25 by 10 by 5 mm., and on being sectioned appeared a homogeneous dark brown. The lymph glands at the hilus of the liver and about the abdominal aorta resembled the peripancreatic nodes.

The bone marrow was moderately firm and light purplish gray.

*Microscopic Examination.*—The hepatic structure was broken up into irregular islands of parenchyma, separated by broad strands of connective tissue. These strands, which radiated from the portobiliary septums, did not break into the islands. They contained branched tubuli, as well as solid cords of cells and a moderate number of blood vessels. In addition to these narrow tubuli, angioma-like areas were found, which were composed of dilated tubuli lined by a very low epithelium and filled by clusters of golden brown, inspissated bile. There were numerous branched or flat and elongated cells, filled by irregular, coarsely granular, deep brown pigment that gave the iron reaction. Round cells of the lymphocytic type and mast cells were also abundant.

The cells toward the periphery of the lobule were small, had shrunken nuclei and were packed with a uniformly granular, light brown pigment. In some instances the nucleus was obscured by the pigment. In the center of the lobule, the cells were larger and had more cytoplasm and larger and more vesicular nuclei.

Here, too, a uniform light brown, granular pigment was present in most instances, but less in amount, decreasing as the center of the lobule was approached; in some lobules, the central portion was entirely free from pigment.

In the cells with scanty pigment, this was found toward the periphery of the cell, while a lighter brown pigment could be discerned around the nucleus. The

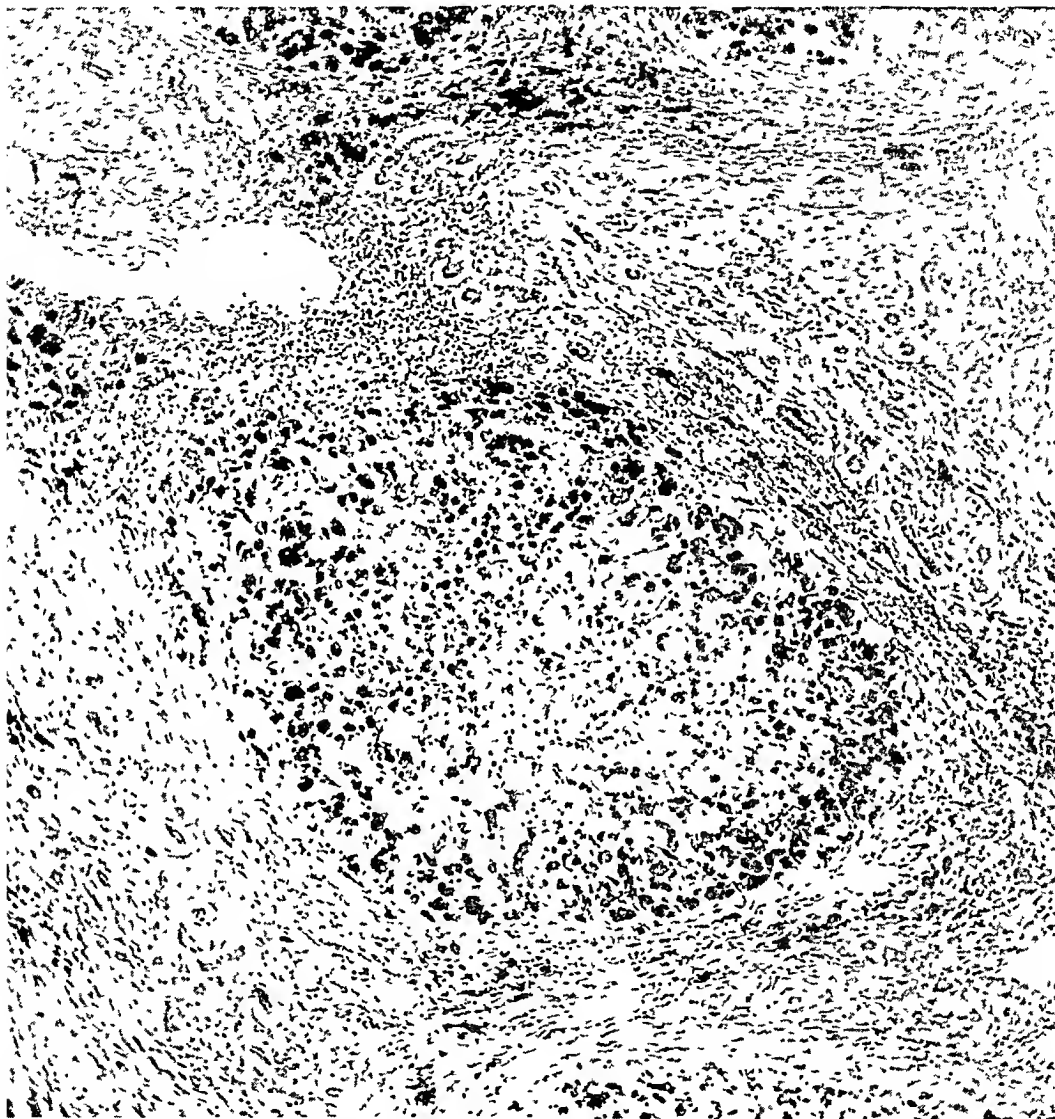


Fig. 1.—A nodule of a cirrhotic liver, showing deposits of iron in the cells of the periphery; iron stain.

peripheral pigment gave the reaction for iron, while that about the nucleus did not. In the lobules in which all of the hepatic cells were shrunken, iron pigment was present in large quantities throughout. It appeared, then, that the more degenerated the cell, the more iron pigment it contained.

The Kupffer cells also varied in their content of pigment. Those in the center of the lobule, in many instances, were free from pigment, while those toward the periphery were saturated with it. Where the hepatic cells contained small



quantities of pigment, the Kupffer cells contained none or a very small amount, but where the hepatic cells contained enormous quantities, the Kupffer cells did also. This was especially true in places where it appeared as if the hepatic cells had been entirely replaced by iron pigment and had lost their cell membranes.

The endothelium of the larger veins contained iron pigment.

With Giemsa's stain, in addition to the iron pigment, there was another type of pigment which formed coarse granules, did not give the iron reaction and

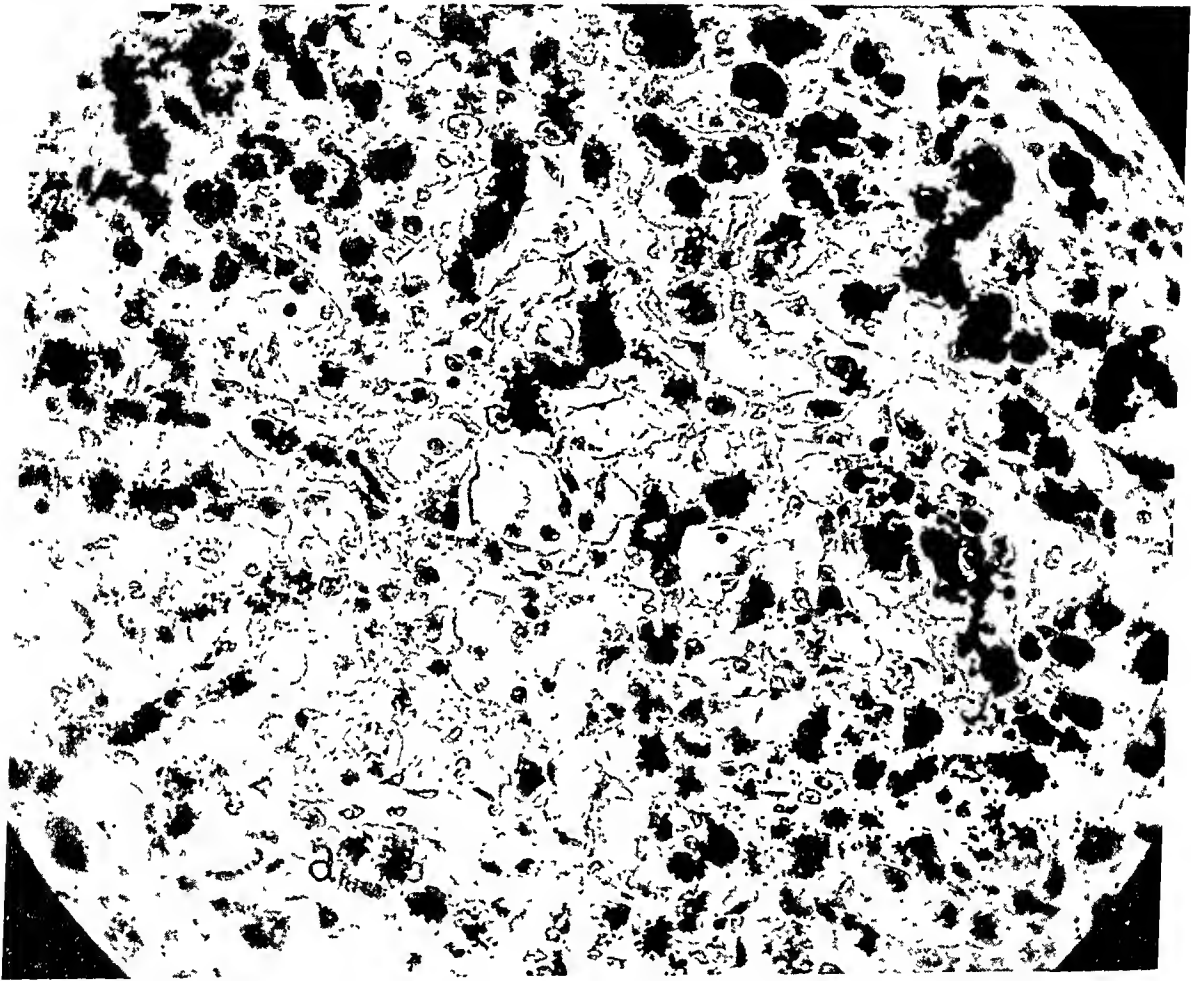


Fig. 2.—A higher magnification of the central portion of figure 1. Note the decrease in iron toward the center of the lobule. The letter *a* shows liver cells filled with iron, while the Kupffer cells are free from it; iron stain.

showed a distinct affinity to the methylene blue. In the hepatic cells this pigment was scanty and restricted to single cells in the periphery of the aforementioned islands of parenchyma and adjacent to the septums. This pigment was located about the nuclei. In the septums the pigment was located in cells that were quite large and had thick branches. These cells were wedged in between the connective tissue bundles, and were most numerous in the adventitia of the smaller arteries. The pigment was also present in the muscle fibers of the media of the smaller arteries, either alone or with iron pigment.

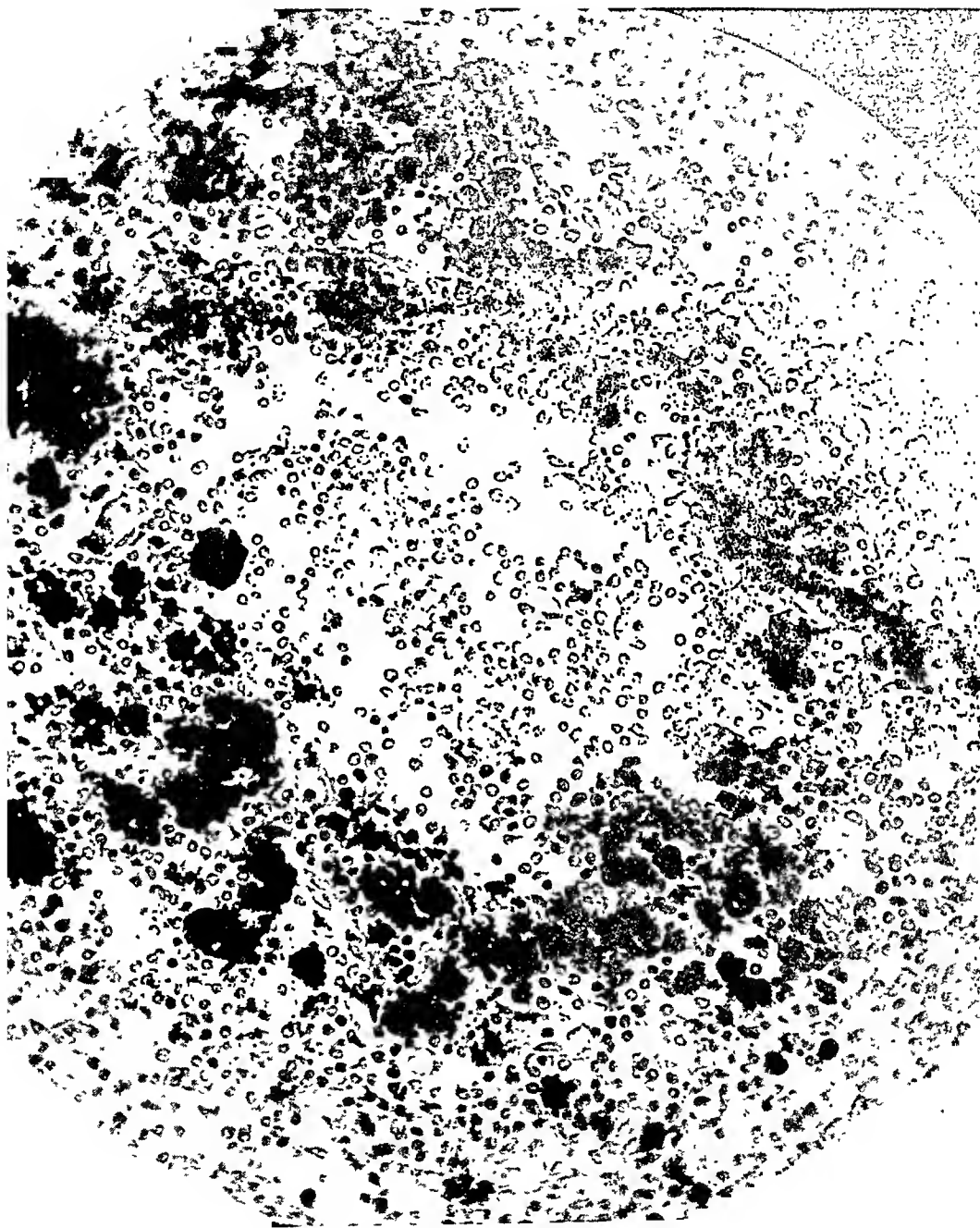


Fig. 3.—A perihepatic lymph gland. The sinuses are filled by huge histiocytes containing iron; iron stain.

In the epithelium of the bile ducts no pigment could be detected.

The tumor was divided by deep fibrous bands into alveoli of polygonal cells with ample, finely granular cytoplasm containing numerous fat droplets and large irregularly shaped nuclei. The nuclei had a moderate amount of chromatin and occasionally oxyphilic nucleoli. In places the tumor showed marked regressive changes, and in places was being transformed into structureless masses. The

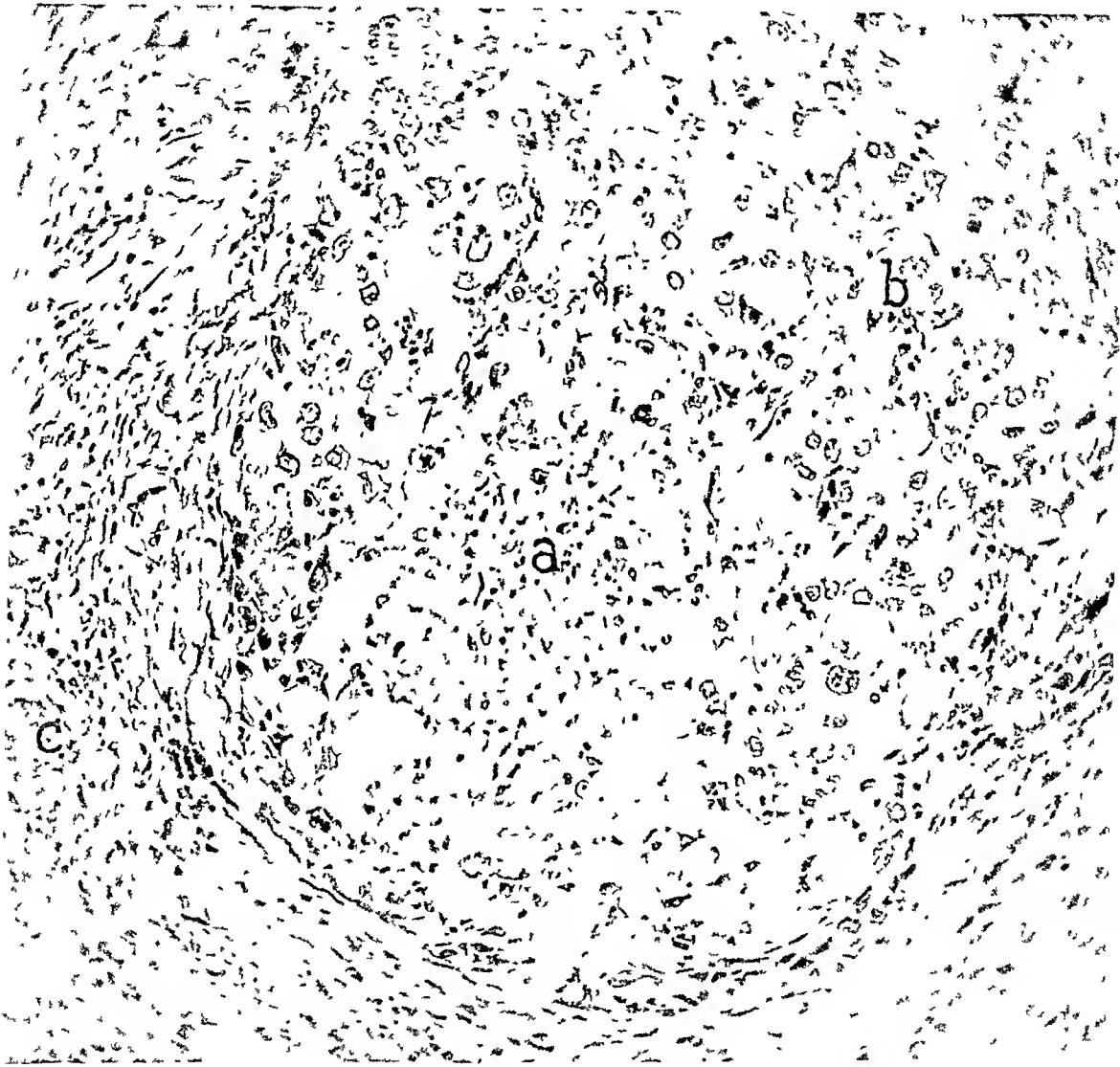


Fig. 4—Hepatocellular carcinoma. The letter *a* indicates a small island of hyperplastic liver cells; *b*, hepatocellular carcinoma; *c*, a bile duct. This section shows clearly the transformation of hyperplastic liver cells into carcinomatous ones. Note the similarity of figures 1 and 4.

portal vein was invaded and filled by tumor cells. No pigment was noted in the carcinomatous cells, although iron pigment was noted in elongated cells of the septums.

The lobules of the pancreas were separated by an increased amount of fat tissue, and groups of fat cells could also be found inside the lobules. There

seemed to be a slight increase of the intralobular stroma, so that the acini were more sharply separated from one another than normally. The glandular cells contained a moderate amount of iron pigment, which was found in the cytoplasm near the lumen of the acinus. The acinar structure was fairly well preserved.

The islands were large and numerous; their cells, too, contained iron, although some of the cells were free from it. The stroma of the islands was not increased. Numerous iron pigment carrying cells were found in the interlobular stroma and about the blood vessels. These cells were either oval or spindle shaped. Another type of cell, elongated with blunt processes, contained light grayish-brown granules that did not give the reaction for iron. When located in the adventitia of the blood vessels or in the walls of the ducts, these cells were rather large. The endothelium of the blood vessels contained no iron.

In the peripancreatic lymph nodes, the interfollicular cords contained an enormous number of iron-filled cells, which formed broad septums, widely separating the follicles. Similar cells had accumulated in the center of the follicles, and by fusion of the centrollicular and perfollicular deposits of iron, the follicles were finally broken up into small islands of lymphocytes. In these islands, too, the reticular cells contained fine iron granules extending into the finest branches, and one could see that the free iron-containing cells broke loose from the "iron-reticulum." The sinuses were inconspicuous.

In addition to the iron-containing cells and small and large lymphocytes, there were many mast cells and plasma cells. The latter took up iron granules, but under the influence of the stored metal seemed to undergo regressive changes. The cytoplasm lost its deep basophilic coloration and assumed a light pink color. The nucleus shrank, and the characteristic chromatin granules became separated from one another. There were free fuchsinophilic bodies and single neutrophilic granulocytes.

The capsule and the trabeculae were thickened and contained iron-laden cells, as well as large oval or elongated cells filled with grayish-brown granules that did not give the reaction for iron, but showed a marked affinity to the methylene blue of the Giemsa solution. The collagenous fibers stained a diffuse blue after Perl's method.

The changes in the peribiliary nodes were similar. Some of the iron-containing cells also contained erythrocytes. In one place there were coarse, branched and segmented trabeculae of a highly refractive iron pigment to which occasionally were attached foreign body giant cells. Some of the giant cells contained iron pigment.

In the tracheobronchial nodes there was much iron pigment, although distinctly less than in the abdominal nodes. The sinuses were discernible, and the reticular structure of the endothelial cells was prominent. They, too, contained iron, but much less than did the reticular cells of the lymphatic tissue proper.

The relation between coal pigment and iron pigment was as follows: Usually there were separate nests of siderocytes and anthracophores, but occasionally both pigments occurred in the same cells.

In the spleen, the sinuses and cords were well separated from each other. The sinuses were of medium width; their lining was flat and contained, occasionally, fine iron granules. In the lumen were mononuclear cells with an ample cytoplasm and fine iron granules, usually near the nucleus. In the cords the reticular fibers were thickened. The meshes of the reticulum were filled with erythrocytes, espe-

cially surrounding the follicles. There were many neutrophilic myelocytes and leukocytes. Small groups of normoblasts, single plasma cells and a few mast cells were present. The reticulum cells, sessile as well as mobilized, contained a moderate number of iron granules, but the majority of the cells were free from iron. Iron stains, however, revealed a heavy incrustation of iron on the connective tissue fibers of some of the trabeculae and on the elastic coats of the arteries. There were many cells filled with iron granules or with a light brown pigment that stained light blue in Giemsa preparations, but did not give the reaction for iron. The cells with the latter type of pigment were larger than the siderocytes.

The follicles were composed of small lymphocytes. In the outer zone there were larger lymphoid cells, and between these cells there were single neutrophilic leukocytes and myelocytes. Scattered about were numerous bone marrow giant cells.

In the reticulum cells of the bone marrow there were single pale erythrocytes and relatively few iron granules. The islands of blood formation separated by fat tissue were in the relation of 1:4. The differential count of the active islands (by Dr. Jaffé) was: myeloblasts, from 6 to 8 per cent; neutrophilic myelocytes, 19.2 per cent; mature neutrophils, 4.6 per cent; eosinophilic myelocytes, 2.8 per cent; mature eosinophils, 6.2 per cent; erythrogonia, 3.4 per cent; erythroblasts, 2.8 per cent; normoblasts, 17.2 per cent; megakaryocytes, 0.2 per cent; plasma cells, 3.8 per cent.

The myocardium showed much pigment accumulated about the nuclei of the muscle fibers. This pigment was light brown and showed an affinity to the stain sudan III, but did not give the reaction for iron. However, more distal from the nuclei were darker brown pigment granules, which in the Perl-stained sections appeared light blue. In the capillaries of the stroma there were occasional iron-carrying histiocytes. There was no pigmentation of the capillary endothelium with iron. The cross-striations of the muscle fibers were fairly well preserved. The intima of the medium-sized arteries was thickened, but free from fat.

In the lung, the alveolar spaces and the alveolar septums contained a moderate number of cells filled with iron and carbon granules. There were a few cells, also, that contained only the iron granules. Anthracophores, free from iron, were more numerous. The alveoli were dilated, and the small bronchi often showed epithelial metaplasia. There was no pigment in the bronchial epithelium.

In the mucosa of the colon there were many round and oval cells that were filled by rather coarse, yellowish-brown pigment granules that did not give the reaction for iron. In addition to the pigment cells, there were many plasma cells. The muscle fibers of the muscularis propria did not contain pigment.

In the skin the melanin content of the basal cells was not markedly increased. In the cutis were small perivascular round cell infiltrations, many plasma cells and single large cells filled with brownish iron-free pigment, which could be differentiated from the melanin.

In the muscle fibers of the submucosal part of the wall of the gallbladder were numerous light grayish-brown pigment granules.

The suprarenal glands, kidneys, prostate and aorta contained no abnormal pigmentation.

*Anatomic Diagnosis.*—The anatomic diagnosis was: hepatocellular carcinoma of the liver in the region of the falciform ligament, with spontaneous rupture and

extensive hemorrhage into the abdominal cavity; tumor thrombosis of the portal vein and metastases to the left lobe of the liver; hemochromatosis with deep pigmentation and cirrhotic changes of the liver and the pancreas, and deep pigmentation of the periaortic, peribiliary and peripancreatic lymph nodes; light yellowish-brown discoloration of the skin, especially of the face; chronic swelling of the spleen with anemia; marked ascites; hypostatic pneumonia of the left lung, and catarrhal colitis with pseudodiverticula.

#### SUMMARY OF STRUCTURAL CHANGES

The most advanced changes were in the liver, which was similar to that found in periportal cirrhosis. The irregular lobules had lost their normal cellular arrangement, with displacement of the central vein and atrophy and degeneration of the cells toward the periphery of the lobule. The amount of iron pigment in a given cell depended largely on the state of the cell; i. e., the more degenerate the cell the more pigment it contained. Thus the most pigment was found in the cells of the periphery of the lobule. At times, oversaturation with iron caused a dissolution of these cells, with liberation of the pigment. It was in these areas that the Kupffer cells and the histiocytes of the periportal areas were most abundant in iron pigment.

The regeneration of the biliary tubuli and hepatic cells was marked, and in the left lobe a malignant transformation of the hepatic cells had taken place. The tumor was subdivided into irregular lobules similar to the nonmalignant portion of the liver. In many instances, the tumor had broken into the portal vein and this was probably the source of the hemorrhage.

The regenerating cells, as well as the tumor cells, were free from iron.

The other pigment-containing organs were for the greater part near the liver; the farther remote, the slighter the changes. Thus the perihepatic and the peripancreatic lymph nodes contained huge amounts of iron pigment, the pancreas less iron and the spleen the least. It was of interest to follow the course of the pigment to the peritracheal glands and finally in the myocardium.

The second type of pigment, the so-called hemofuscin, was found sparingly in the cells of the periphery of the hepatic lobules, in large, elongated cells of the portobiliary septums, in the pancreas, in the spleen, in the myocardium, in the skin and in the chronically inflamed colon and gallbladder. Except in the colon, gallbladder and skin, this light brown pigment was found in association with iron.

As an incidental finding, the storage of iron by the plasma cells in the periportal lymph glands was associated with marked regressive

changes in these cells. The storage of iron is foreign to them, but as the lymph nodes were oversaturated with the pigment, all elements contained it.

#### IRON METABOLISM IN RELATION TO HEMOCHROMATOSIS

Before one can delve into the pathogenesis of hemochromatosis, some of the newer concepts and the experimental data concerning iron, copper and zinc metabolism must be briefly presented.

Starkenstein showed that, normally, ferrous iron is absorbed in the stomach and duodenum, oxidized in the blood to ferric iron and reduced in the liver to ferrous iron, where it enters into the formation of hemoglobin or is excreted. The endogenous iron derived from tissue catabolism and from hemoglobin is stored in the spleen (Schmidt). The excretion of iron takes place largely by way of the intestine and to a small extent by way of the kidneys.

Schultze verified Starkenstein's observations by showing that in rats, normally, the liver contains 13 per cent ferric iron and 86.7 per cent ferrous iron, while the spleen contains 44 per cent ferrous iron and 66 per cent ferric iron.

Intravenously injected colloid and molecular aspersions of iron has a great affinity for the Kupffer cells of the liver (Eppinger, Wegener, Jaffé). The liver cells, too, take up the iron when large amounts are injected (Eppinger, Wegener). The injected iron acts as a vital stain, but cannot be considered as duplicating the normal mechanism of iron metabolism. It does simulate, however, the liberated iron in hemolytic anemias and hemosiderosis from other causes (Rössle, Hauser, Rous and Oliver, Wegener).

In hemochromatosis, as shown by Rössle, the process is reversed, and as described in the case reported here, the liver cells in the center of the lobule contain iron, while the Kupffer cells do not. This would indicate that the iron first appears within the liver cells, and that only after saturation or dissolution of these cells do the Kupffer cells take up the pigment.

The mechanism by which the hepatic cells obtain the iron, as suggested by Rössle and later modified by Eppinger, could not be verified by me or by other investigators writing on the subject. Rössle noted in a pigmented liver the destruction of red blood corpuscles by the liver cells proper, and Eppinger explained this phenomenon by a hypofunction of the Kupffer cells that allowed the red cells to break through their barrier. However, the absence of anemia in hemochromatosis has been repeatedly shown (Elmer, Parker, Eppinger). An active bone marrow or an increase in the bilirubin of the bile or in the urobilin of the feces has not been demonstrated (Althausen and Kerr).

The iron, then, is not derived from the hemoglobin, for when it is considered that there is from twenty to one hundred times as much iron in the body in hemochromatosis (Wegener, Howard and Stevens, Muir and Dunn) as is found normally, one would expect a severe anemia or a hyperactive bone marrow to supplement the marked destruction of blood.

The source of the iron has been a matter of much dispute. Garrod found that in a case of hemochromatosis there was a complete retention of food iron (normally 11 mg. per day), as he was unable to recover iron from the feces, urine or bile. Howard and Stevens found only a slight retention of iron and reported only 2.5 mg. retained over a period of five days. What probably occurs is that there is a variation in the retention and excretion of iron, similar to that in nitrogenous retention in the exacerbations and remissions of nephritis. With a calculated mean of 6 mg. per day of iron retained, it would take from eight to twelve years for the disease to develop. This is compatible with the reports of cases known to have been cases of hemochromatosis. Further investigations along these lines are essential.

Whether endogenous iron plays a rôle is doubtful because of the absence of anemia and because, as Schmidt has shown, iron derived from the catabolism of tissue and from hemoglobin is mainly stored in the spleen.

The question then arises: At what station in the metabolism of food iron are abnormal manifestations evident? Chemical determinations have shown that in hemochromatosis the iron of the blood is slightly decreased (48 mg., according to Garrod, and 45 mg., according to Howard and Stevens, as compared with a normal of 54.5 mg., according to Fowell). The fault must lie with the liver, in its failure to excrete the iron. This hypofunction is probably the result of an inability to reduce ferric to ferrous iron, which is the only form in which the body can utilize it.

I suggest that if at operation hemochromatosis is diagnosed, a specimen of the liver be taken for a determination of the ferrous and ferric iron contents, as after death the ferrous iron is gradually oxidized to ferric iron (Schultze).

The fact that zinc, which is more difficult to excrete than iron, is not retained in the liver can perhaps be explained by the fact that the necessity for reduction of the zinc before its excretion has not been demonstrated.

#### COPPER METABOLISM IN RELATION TO HEMOCHROMATOSIS

Since Mallory suggested chronic copper poisoning as a possible source of hemochromatosis and believed that he had produced this



condition in rabbits and monkeys by feeding them copper salts, much interest has been aroused regarding the rôle of copper.

Normally, the amount of copper in the liver varies considerably. Schönheimer and Oshima reported as normal from 1.3 to 3.9 mg.; Herkel, from 6.5 to 36.1 mg., and Kleinmann, from 22.7 to 48.7 mg. (men). Schönheimer reported from 9.86 to 63.2 mg. of copper in the liver in hemochromatosis, and Herkel, from 42 to 384 mg. However, Kleinmann showed that in still-born infants and in infants up to 3 days old the amount of copper in the liver varies from 324 to 516 mg. The amount decreases rapidly, so that in children from 13 weeks to 2 years old, there is only from 12 to 24.4 mg. of copper. Cirrhosis or hemochromatosis of the liver was not evident in any of his cases. Herkel found that in pregnancy, also, the amount of copper is doubled (from 13 to 72 mg.), while in nonpigmented cirrhosis the amount of copper can vary from 67.6 to 379 mg.

These results and the inability of the majority of investigators to produce hemochromatosis by the feeding or by the injection of large quantities of copper (Polson, Flinn and Von Glahn, Wegelin, Herkel, Lubarsch) fail to verify the conclusions of Mallory, and discount the rôle of copper in the formation of hemochromatosis.

How can one explain the increase of copper in hemochromatosis? McHargue reasons that with the growth of an animal or man copper is increased, yet in tumor of the liver there is a decrease in the amount of copper in the tumor proper (Keith and McNair). In our material the copper content of the primary tumor of the liver was not elevated (15 mg.), while the amount of copper in the nonmalignant portion was increased (80 mg.).<sup>1</sup> Thus one cannot conclude that the regeneration of hepatic tissue is responsible for the increase in copper.

Hart, Steenbock, Waddell and Elvehjem proved that in rats iron alone will not relieve anemia, but must be associated with copper. The latter, however, does not enter into the formation of hemoglobin, as hemoglobin is free from copper. The copper merely acts as a catalytic agent as in the production of the noniron-containing chlorophyll molecule.

Can one deduce from the aforementioned experimental data that, because copper and iron are both necessary to the formation of hemoglobin, the iron in the body may have a definite affinity for the copper? If so, the increase in the copper of the liver in hemochromatosis can be explained by the corresponding increase in the iron. The large amounts of copper in still-born and new-born infants would be explained by the active erythropoiesis in the former and the increased destruction of

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1. The copper determinations were carried out in the laboratory of Dr. Rudolph Schoenheimer, Freiburg, Germany. All values are for 1000 Gm. of liver tissue.

blood in the latter. In pregnancy, the increase in copper may be derived from the fetus. In simple cirrhosis without hemochromatosis, the increase in copper is due to a corresponding increase in the iron.

The relationship between copper and hemochromatosis is therefore only secondary, being dependent on the iron content of the liver.

#### HEMOFUSCIN AND HEMOCHROMATOSIS

The origin of hemofuscin, the second type of pigment found in hemochromatosis, is more obscure than that of hemosiderin. It is generally accepted that hemofuscin is not an iron-free blood pigment, i. e., hematoidin. Many authors, however, feel that it is an intermediate product between hemoglobin and hemosiderin (Mallory, Parker and Nye, Eppinger, Wegener, Hueck), and Muir and Dunn reported that if hot hydrochloric acid is used, instead of cold hydrochloric acid, in the Perl reaction for iron, the hemofuscin takes a blue color similar to that of hemosiderin. However, it must be borne in mind that with the use of hot hydrochloric acid the cells may be so injured as to liberate the combined iron that normally does not give the reaction for iron (Wells). Furthermore, as has been stated, anemia or active proliferation of the bone marrow is uncommon with hemochromatosis, and hemolysis of the red blood cells has not been shown (Funk and St. Clair).

Because hemofuscin is found rather diffusely and not necessarily associated with hemosiderin, it is more likely that the former is the result of an irritation of the cell produced by the same toxin that is responsible for hemochromatosis.

In the case reported, hemofuscin alone was found in the skin and musculature of the gallbladder and in the intestines, while in the other organs it was associated with hemosiderin. The concept that hemosiderin is always present in the skin in hemochromatosis is erroneous, and although, when present, it is fairly diagnostic, its absence does not exclude hemochromatosis. More commonly hemofuscin is found alone (Rolleston).

#### PATHOGENESIS

The isolation of the causative agent in hemochromatosis is not within the scope of the pathologist, but the pathogenesis may be determined by him, as much can be learned from histologic studies.

The underlying physiologic abnormality is probably the inability of the hepatic cells to reduce ferric iron to ferrous iron, the only form in which iron can be utilized by the body.

A possible mechanism by which this was brought about in the case reported was as follows: The low grade chronic infection in the colon, decreased the bactericidal and detoxifying powers of the intestinal mucosa

and allowed various toxins and even bacteria to enter the portal circulation. The hepatic cells in the periphery of the lobule were so injured as to impair its power of reducing iron, so that the iron accumulated in these cells. On saturation of the cells with the pigment, the nuclei became shrunken, and finally the cells were destroyed, liberating the iron pigment. The Kupffer cells of the liver in these areas and also the resting histiocytes of the periportal areas, phagocytosed this iron.

Toward the center of the lobules, the concentration of the toxin was diminished, and thus only slight changes in these cells were noted. These cells contained only a small amount of pigment, while the corresponding Kupffer cells contained a lesser amount or none. These observations tend to show that the liver cells were the first to contain the iron, and that only on saturation or dissolution of these cells, with liberation of the pigment, did the Kupffer cells take up the iron.

The progressing degeneration of the hepatic parenchyma, especially that at the periphery of the lobule, and its replacement by fibrous tissue led to periportal cirrhosis. The regeneration of the liver cells became excessive and at length autonomous, resulting in a malignant transformation of these cells (Goodpasture, Oertel, Winternitz, Jaffé, Rolleston).

With the liberation of huge quantities of iron in the liver, the draining lymph glands joined in the storage of it. In a similar manner, the pancreas, the spleen, the peritracheal lymph glands and the myocardium contained iron, but the organs proximal to the liver contained the most, and those distal, the least. The amount of iron in any tissue was directly proportional to its proximity to the liver.

Because of the large amounts of iron in the liver, the amount of copper was correspondingly increased, perhaps as a result of a definite affinity of the iron for copper in the body. That no anemia resulted was due to the fact that there was sufficient parenchyma in the center of the lobules to carry on the formation of hemoglobin.

The toxin or bacteria liberated from the colon also affected the cellular elements in other parts of the body, irritating them and causing thus the formation of hemofuscin, a degenerative product of the cell.

The presence of iron in the epithelium lining the glands of the pancreas, the thyroid gland and other glands may mean that these organs, too, have a rôle in iron metabolism, and that the hypofunction of the cell as a result of its intoxication may hinder it from metabolizing the iron.

#### CONCLUSIONS

A case of generalized hemochromatosis is reported, complicated by a primary hepatocellular carcinoma of the liver, which had eroded a large hepatic vein and produced death by intra-abdominal hemorrhage.

From the histologic studies it was concluded that the liver cells in hemochromatosis are the first to contain iron and that only after saturation or dissolution of these cells do the Kupffer cells take up the pigment. The amount of iron pigment in other organs was directly proportional to their proximity to the liver. The increased amount of copper in the liver is explained by a definite affinity of the iron for copper in the body.

Cirrhosis of the liver in association with hemochromatosis does not predispose to carcinoma more frequently than cirrhosis of the liver alone.

It is suggested that the inability of the liver cells to reduce ferric to ferrous iron is the underlying physiologic abnormality in hemochromatosis, and further studies to determine the percentages of ferrous and ferric iron in the liver are urged. These determinations must be carried out on fresh specimens by biopsy or shortly after death, as the ferrous iron is gradually oxidized to ferric iron postmortem.

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# A COAGULOFLOCCULATION TEST FOR MALIGNANT TUMORS\*

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Many attempts have been made to utilize the blood serum of patients with malignant tumors for diagnostic purposes. In a few instances, the fundamental idea was that malignant tumors or their products may form antibodies similar to those of bacterial origin. A number of methods are founded on some differences in the protein fractions between the serums of patients with malignant tumors and the serums of normal persons. The principles on which some other procedures are devised include changes of properties of the blood in malignant conditions, with regard to (1) hemolysis, (2) viscosity, (3) ferments, (4) coagulation, (5) color reactions with some dyes, (6) sedimentation of red cells and (7) the blood constituents.

The reasons why most of the tests have found only a limited use and recognition may be many: (1) The principle on which the method is based may apply to the blood only in a certain number of cases of malignant tumors; (2) the blood from a certain percentage of normal persons may show the same properties, which would render the method of doubtful value; (3) the method may be technically too complicated to be of practical value; (4) the procedure may be too sensitive; (5) it may not be sufficiently sensitive.

The method described now was developed as the result of a careful comparison of the principles, technic and results of other methods and study of various chemical and serologic properties of blood of apparently normal persons and patients with malignant tumors and other diseases. My observations seem to indicate that the serum is more useful for this purpose than any other constituent of the blood. Some important serologic factors, such as antigen, diluents, temperature and duration of incubation, seem to have a great bearing on the sensitiveness of serums of patients with malignant tumors. The term "malignant serum" will be used in this paper in a sense similar to that in which the term "syphilitic serum" is used.

In devising the procedure, special attention was paid to the factors that usually determine the extent of the use of a test, namely: (1) simple technic, (2) shortness of time required for the test, (3) easy preparation of reagents, (4) elimination of inactivation of serums, (5)

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highest possible sensitiveness without rendering normal serums or those from other diseases positive.

### EQUIPMENT

*Glassware and Apparatus.*—For the measuring of serum and antigen, 0.2 cc. pipets are required. For the dilution of the serum, 1 cc. serologic pipets are most satisfactory. Wassermann tubes with a diameter of  $\frac{1}{2}$  inch (1.27 cm.) and 4 inches (10.16 cm.) long appear to be very suitable. Similarly, tubes of the same diameter, but 2 inches (5 cm.) longer, are useful in instances in which deeper submersion in the water bath is necessary or more desirable. The glassware should be rinsed with warm water and placed over night in a cleaning solution (equal parts of a 6 per cent solution of sulphuric acid and a 6 per cent solution of sodium or potassium dichromate), then rinsed several times with distilled water and allowed to dry. Racks for Wassermann tubes with two or three rows of holes are most useful. A water bath easily adjustable to from 54 to 55 C. and maintaining a uniform temperature is required. An interval timer with an automatic alarm is convenient on account of the brief incubation.

*Blood Serum.*—Serums should be fresh and thoroughly centrifugated; they do not require inactivation. Serums that are rich in lipoids, contaminated or hemolytic should be eliminated as unsatisfactory for the test.

*Serum Diluent.*—Distilled water is used instead of salt solution.

*Antigen.*—Plain alcoholic beef heart antigen: Beef heart is freed from all fat and finely ground. One hundred grams are extracted with 1,000 cc. of 95 per cent alcohol for three days at 37 C. and then left over night at room temperature. The extract is then filtered and kept in dark bottles, closed with rubber corks. The antigen is then ready for use.

*Sodium Chloride Solution.*—Sodium chloride solution is prepared by dissolving 40 Gm. of chemically pure sodium chloride in 130 cc. of distilled water. A saturated solution of sodium chloride is equally satisfactory, if filtered before it is used.

### PROCEDURE

*Method of Calculating Dilutions of Serums Previous to Use in Test.*—The dilution of serums is carried out according to the percentages of hemoglobin. Tallqvist's scale is most commonly used in the determination of hemoglobin; it is disadvantageous because it is less accurate than other procedures. In order to have uniformity in the results obtained, Dare's hemoglobinometer is used as a standard. To the percentage of hemoglobin obtained with Dare, 10 is added, and the sum is divided by 20, which gives the dilution for the respective serum. (For instance, if the reading is 70 per cent, 10 is added, giving 80. This divided by 20 equals 4; the dilution of the serum in this case would be 1:4). Tallqvist's scales can be used if their average reading is previously compared with Dare and the difference taken into consideration in the calculation of the dilutions of the serums. (For instance, if the Tallqvist scale shows a 10 per cent higher reading than the Dare, nothing is added to the hemoglobin reading before dividing with 20. If the Tallqvist reading is 15 per cent or 20 per cent higher than Dare, 5 or 10, respectively, is subtracted from the reading before dividing with 20). When the Dare reading is 40 per cent or less, the serums should be diluted only to 1:2.5.

*Titration of Antigens Previous to Use in the Test.*—The selection of the proper amount of antigen is based on its reaction with malignant and syphilitic serums. The syphilitic serums are more sensitive than the normal serums and are there-



fore more valuable for the titration. Properties similar to those of syphilitic serum are observed also in the serum in jaundice. All antigens are titrated in the following manner: In each of two rows of a rack, eight tubes are placed. Increasing amounts of undiluted antigen (0.12, 0.14, 0.16, 0.18, 0.20, 0.22, 0.24, 0.26 cc., etc.), are placed in the corresponding tubes of both rows. In each tube of the first row, 0.6 cc. of the diluted (according to the procedure described) malignant serum is added, and each tube of the back row receives 0.6 cc. of the similarly diluted syphilitic serum. The tubes are thoroughly shaken and then placed in a water bath for five minutes at from 54 to 55 C. After the incubation, the content of each tube is slowly diluted with 2.5 cc. of saturated sodium chloride solution, and the results are recorded. Usually the smaller amounts of antigen cause turbidity in the syphilitic and malignant tubes, "lower nonspecific zone." Larger amounts cause turbidity in the syphilitic tubes, while in the malignant tubes the more or less coagulated serum floats on the surface of the saline solution, which contains floccules. This is the "specific zone." Still larger amounts of antigen cause a positive reaction (flocculation) both in the malignant and in the syphilitic tubes, "upper nonspecific zone." The largest amount of antigen that causes only turbidity in the syphilitic tube and a distinct coagulo-flocculation in the malignant tube is selected as the proper amount for the test ( $\equiv$  titer). The titrated amount of antigen should also be tested with icteric and anemic serums. The titer remains the same for an indefinite period if the antigen is properly preserved.

*Routine Test.*—Wassermann tubes are placed in two rows in the racks. The tubes of the first row are used for the main test with the unknown serums and also for the malignant, syphilitic, icteric and anemic controls. The last tube in the first row contains the antigen control. The tubes in the second row serve as the serum controls for the unknown serums and also for the malignant, syphilitic, icteric and anemic serums. The titrated amount of the undiluted antigen is placed in each tube of the first row. The corresponding amount of distilled water is placed in all tubes of the second row. Six tenths of a cubic centimeter of each diluted serum is added to one tube in the first row and an equal amount of the same serum to the tube behind in the second row. Six tenths of a cubic centimeter of distilled water (instead of serum) is added to the antigenic control. The ingredients of malignant, syphilitic and icteric controls and their serum controls should be the same as those used for the unknown serums. All tubes are then shaken and placed in a water bath at from 54 to 55 C. for five minutes. After the incubation, 2.5 cc. of a saturated, or 32.5 per cent sodium chloride solution is slowly added to each tube, and the results are read.

If the required amount of the unknown serum is not available, the test may still be performed successfully if the remaining constituents for the reaction are decreased proportionately.

*Controls.*—The following controls are necessary each time the test is carried out: (1) antigen control, (2) serum control (each serum should have a serum control), (3) malignant, syphilitic, icteric and anemic controls.

*Interpretation of the Results.*—The controls should be examined before making readings of the unknown serums. The malignant control should show a thick layer of coagulated serum floating on the surface of the salt solution, which contains many large floccula. All other controls should remain uniformly turbid. One tube is read for each unknown serum. Tubes showing the same reaction as the malignant control are read as strongly positive. Tubes with a distinct flocculation without showing in addition a layer of suspended coagulated serum on the surface of the saline solution are read as weakly positive. Uniformly turbid tubes are

read as negative. Tubes with a doubtful flocculation are also read as negative and the test should be repeated.

*Sources of Error.*—(1) The used serum is hemolytic, contaminated or inactivated, or contains an excessive amount of lipoids; (2) the serum is not properly diluted; (3) some other diluent was used instead of water; (4) the antigen was not properly prepared or preserved; (5) the antigen was not accurately titrated, thus causing either nonspecific reactions or a low percentage of specific reactions; (6) the antigen was not vigorously mixed with the diluted serum; (7) fallacies occurred

TABLE 1.—*Malignant Tumors*

Kind and Location	Cases	Positive Reaction		Negative Reaction
		Strong	Weak	
<b>Carcinoma</b>				
Breast.....	21	16	3	2
Cervix.....	45	38	5	2
Cheek.....	1	..	..	1
Colon.....	8	6	..	2
Ear, exterior.....	1	1	..	..
Epiglottis.....	1	1	..	..
Esophagus.....	5	4	1	..
Eye.....	1	..	..	1
Jaw.....	2	2	..	..
Kidney.....	1	1	..	..
Larynx.....	12	8	..	4
Lip.....	3	2	..	1
Liver.....	2	2	..	..
Mandible.....	1	1	..	..
Mouth.....	2	2	..	..
Neck.....	2	2	..	..
Orbit.....	1	..	..	1
Palate.....	1	1	..	..
Pancreas.....	1	1	..	..
Peritoneum.....	1	1	..	..
Prostate.....	1	1	..	..
Rectum.....	13	8	..	5
Scalp.....	1	1	..	..
Serotum.....	1	1	..	..
Sinus, frontal.....	2	2	..	..
Sinus, maxillary.....	3	3	..	..
Stomach.....	12	9	..	3
Tongue.....	3	3	..	..
Tonsils.....	4	3	..	1
Uterus.....	16	12	2	2
Vulva.....	2	2	..	..
<b>Sarcoma</b>				
Lung.....	1	1	..	..
Neck.....	1	1	..	..
Thigh bone.....	1	..	..	1
<b>Melanosarcoma</b>				
Skin.....	3	3	..	..
<b>Hodgkins disease</b>				
Neck.....	3	3	..	..
<b>Total.....</b>	<b>179</b>	<b>142</b>	<b>11</b>	<b>26</b>
<b>Percentage.....</b>		<b>79.33</b>	<b>6.15</b>	<b>14.52</b>

in the reading of the temperature and in the duration of incubation; (8) another reagent than the saturated salt solution was used for the dilution of the serum-antigen mixtures after the incubation; (9) the tubes were shaken after addition of salt solution, thus rendering the reading difficult.

#### STATISTICAL DATA CONCERNING THE SENSITIVENESS OF THE TEST

The blood specimens for the study of this reaction were obtained from patients in the Cook County, St. Anne's and Holy Cross Hospitals in Chicago. For the development of this procedure, about 500 samples

of blood were taken from patients with malignant tumors. In the statistical table of malignant tumors only cases are included in which the clinical diagnosis was confirmed either by biopsy or by necropsy (179). Most of the cases of malignant tumors with a negative reaction represent either early stages or very advanced stages with a high degree of cachexia. The cases of benign tumors include: 5 lipomas of the back, 1 papilloma of the bladder, 15 adenomas of the breast, 2 fibromas of the ovaries, 4 dermoid cysts of the ovaries, 8 serous cystadenomas of the ovaries, 43 simple cysts of the ovaries, 2 mixed tumors of the

TABLE 2.—Other Diseases

Kind of Disease	Cases	Positive Reaction		Negative Reaction
		Strong	Weak	
Aneurysm of the aorta.....	1	1	..	..
Angina ludovici.....	1	..	..	1
Appendicitis.....	20	..	..	20
Cholecystitis, chronic.....	15	..	..	15
Cirrhosis of liver.....	2	..	..	2
Croupous pneumonia.....	3	..	..	3
Diabetes mellitus.....	6	..	..	6
Diphtheria.....	1	..	..	1
Ectopic pregnancy.....	1	1	..	..
Empyema.....	3	..	..	3
Exophthalmic goiter.....	5	..	..	5
Gastric ulcer.....	4	..	..	4
Jaundice.....	12	..	..	12
Leukemia, lymphatic.....	2	..	..	2
Leukemia, myelogenous.....	1	..	..	1
Measles.....	1	..	..	1
Meningitis, cerebrospinal.....	3	..	..	3
Nephritis, chronic parenchymatous.....	3	..	..	3
Nephritis, chronic interstitial.....	1	..	..	1
Peritonitis, acute.....	7	..	..	7
Pernicious anemia.....	4	..	..	4
Pregnancy.....	32	..	..	32
Pulmonary tuberculosis.....	14	..	..	14
Pyelonephritis.....	1	..	..	1
Renal tuberculosis.....	1	..	..	1
Scarlet fever.....	7	..	..	7
Sclerosis, multiple.....	1	1	..	..
Typhoid fever.....	2	..	..	2
Total.....	154	3	..	151
Percentage.....		1.95		98.05

parotid, 1 hemangioma of the skin, 6 nevi of the skin, 1 teratoma of the testicle and 27 fibromyomas of the uterus. In all the cases of benign tumors, the blood gave a negative reaction.

Of 154 cases of other diseases (table 2), only about 2 per cent gave a positive reaction without clinical evidence of a malignant condition.

## COMMENT

A method has been devised for the serologic diagnosis of malignant tumors. This procedure resembles in many respects the various flocculation tests for syphilis. The antigen is simple in its preparation and does not require the addition of cholesterol. The test offers the further advantage that the serums do not require inactivation. If necessary,

the test can be carried out with very small quantities of antigen and serum. The brevity of incubation is also a convenient factor.

The reaction is caused by the influence of both constituents of the antigen: alcohol and lipoids. It occurs very definitely at a suitable temperature (from 54 to 55 C.). It occurs even if the alcohol does not contain lipoids. The lipoids increase the intensity of the reaction. This is the case only with a certain kind of lipoids present in a proper quantity. Other solvents and lipoids that may be useful for this procedure will be reported on in a subsequent paper. How early the reaction becomes positive, whether the removal of a malignant growth has any influence on the reaction, and whether the recurrence can be reflected in the reaction will be material for further observations. Work to determine to what extent, if any, diseases other than malignant tumors yield a positive reaction is being carried on.

#### SUMMARY

A serologic test for malignant tumors has been devised, which offers the following advantages: The antigen used is simple and easily prepared; the serums do not require inactivation; the duration of incubation is brief (five minutes); the reaction is definite and can be easily interpreted; the test offers a reasonably high degree of sensitiveness and accuracy.

# General Review

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## THE ETIOLOGY OF CANCER

### II. IRRITATION \*

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OMAHA

In 1863 Virchow, discussing the origin of malignant tumors, emphasized the importance of chronic irritation as a frequent, if not entirely essential, cause. Even before that there had been described the close association that may exist between certain types of carcinoma and specific sources of irritation; the exceptionally high incidence of skin cancers in chimney-sweeps had been observed by Pott in 1775; the association between cancer of the lip and pipe smoking, by Soemmering in 1795, and again by Melzer in 1850, and the occurrence of skin cancer as a sequel to lupus, by Devergie in 1857. Similar observations post-dating Virchow's were those of Volkmann in 1885, concerning the occupational incidence of skin cancer in workers in the paraffin oil and tar industries, and the description of kangri-burn cancer by Maxwell in 1879.

The possibilities of the use of some of these apparently specific irritants in the experimental induction of cancer were realized early, and in 1889 Hanau announced that after long-continued painting of the skin of rats with tar no cancer resulted, but merely chronic inflammatory lesions, and 1894 Cazin was likewise unsuccessful with dogs—both, as it chanced, having worked with animals unsuited to this particular method. In 1911 Wacker and Schminke applied tobacco tar to the skin of the rabbit; in 1912 Bayon studied the effect of the subcutaneous injection of tar into the rabbit's ear, in which he got excessive epithelial hyperplasia, but without actual malignancy, and in 1913 Haga applied soot to the skin of that animal; all of these experiments yielded negative results as far as actual causation of malignant growth was concerned apparently because of insufficient time being allowed for them. In the meantime, other specific forms of cancerogenic irritation were being learned from clinical observation. Arsenic, which had been alleged to be causative of cancer in 1820 by Paris, with the statement that scrotal cancer was frequent in employees, and cancer of the rump in cattle, in the mining districts of Cornwall—an observation

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that lacks confirmation—was definitely associated with malignant growth by Hutchinson in 1887. Rehn in 1895 reported on tumor of the bladder in aniline workers, and in 1906 Wyss called attention to the induction of skin cancers by repeated x-ray burns.

To the extent that these clinical observations gave rise to successful attempts at experimental induction of cancers, the first to be confirmed in this way was the effect of the x-rays; in 1910 Marie, Clunet and Raulot-Lapointe were able to announce that they had produced sarcoma in the rat by heavy ulcerative doses. The production of skin cancer in the rabbit by the prolonged application of coal tar was first reported by Yamagiwa and Itchikawa in 1915, although a year earlier they had reported the development of papillomatous lesions by this method. Similar results with mice were announced by Tsutsui in 1918. Owing to the outbreak of the World War and to the fact that their earlier publications were in Japanese, the experiments of Yamagiwa and Itchikawa and of Tsutsui failed to obtain extensive notice until considerably later, and they lacked confirmation from other than Japanese sources until 1920, when similar results were announced by Fibiger and Bang.

#### CHEMICAL IRRITATION

*Tar.*—The method of induction of malignant tumors by the use of tar has proved extraordinarily adapted to experimental study and has given rise to a considerable literature; as this up to 1926 has been reviewed in the ARCHIVES by Woglom, whose article is furnished with an exceptionally complete bibliography, a detailed survey of this phase of cancer research would appear scarcely necessary, and accordingly only the general results will be summarized here.

Although in suitable animals positive results ranging from 100 per cent (Fibiger and Bang) downward have been reported, not nearly all experimental animals acquire tumors on tarring; rats (Hanau, Paszkewicz, Yamagiwa, Polettini, Buschke and Langer, Borrel, Boez, de Coulon, Halberstaedter, Deelman, Teutschlaender, Möller, Dentici and von Puhr) are almost completely resistant to the development of tar cancers of the skin, although 1 success in 100 rats has been reported by Herly, and another somewhat doubtful instance by Cholewa. Fowls (Yamagiwa, Teutschlaender and Choldin) are likewise almost completely resistant to the local action of tar on the skin, although Choldin found that by the cautious application of tar, either pure or in ether solution, he could induce hyperkeratoses and in some cases cancrioid tumors after long periods; guinea-pigs (Menetrier and Surmont, Leitch, Mertens, Itchikawa and Baum, Dentici, Halberstaedter, Teutschlaender, Borrel, Boez, de Coulon and Bloch) and dogs (Itchikawa) all show almost complete insusceptibility on the part of the skin. On the other hand,

rabbits, mice particularly, and of course human beings react with some regularity; in human beings, in addition to the somewhat random action of tar as occurring in gas-house workers and others of like occupations, 2 cases in circumstances almost experimental have been reported, the one by de Jong, Meyer and Martineau, in which skin cancer developed after long-continued painting of an inflammatory skin lesion with tar, the other a somewhat similar instance reported by Veiel, the irritant in this case being pine tar. But although skin cancers occur almost never in tarred rats, fowls, guinea-pigs and dogs, the tumefacient action of tar may be manifested with greater readiness in these animals at the expense of other tissues. Maisin and Picard reported the production of a papillary carcinoma of the bladder in a rat after the introduction of tar embedded in solid material, with continued injection of tar for 30 days thereafter; Menetrier, the appearance of a gastric carcinoma after intraperitoneal injection of tar; Teutschlaender, epithelial metaplasia and a cancer of the horn of the uterus after intravaginal introduction of tar. Sarcomas after subcutaneous injection of tar were reported by Russell, and after inunction, by Badile, who also reported metaplasia and carcinomatous growth after the introduction of tar into the seminal vesicle; Russell and Melczer reported that sarcomas may follow not only subcutaneous injection, but also inunction, of tar—all in rats. And in these animals Buschke and Langer, although they found that only slight local effects followed repeated intrarectal injections of tar, observed with some regularity papillomatous growths in the forestomach, with in some cases what appeared to be epithelial infiltration; a similar result was obtained after the feeding of small amounts.

With fowls, too, malignant neoplasms have been caused elsewhere than in the skin by the use of tar. Sarcomas have been produced in this animal by Carrel, and Murphy and Landsteiner, by the double insertion into adult animals of embryonic fowl tissues and tar, and more indirectly by Laser, by the treatment of cultured embryonic fowl tissues with the plasma of an animal that had been treated by injection of tar. Also the injection of tar alone has occasionally led to connective tissue tumors or other internal neoplastic development, in the hands of Choldin. With the guinea-pigs Kazama obtained the induction of adenocarcinoma of the gallbladder following the introduction of tar in conditions assuring an added element of mechanical irritation. His exceedingly high percentage of positive results might lead to a suspicion of their authenticity, but Leitch, contrary to his expectations, was likewise able to secure carcinomas with great frequency in similar experiments. Kimura reported multiple adenocarcinomas of the bronchi in guinea-pigs 140 days after the insufflation of tar.

In addition to the facile production of skin cancers in mice and rabbits, other tumors may be caused in these animals by the suitable

application of tar. Injected into the breast in rabbits it may cause mammary adenocarcinoma, as announced by Yamagiwa, Suzuki and Murayama, or sarcoma (Yamagiwa); into the gastric submucosa, papillary adenomas (Ishibashi and Ohtani); into the renal pelvis, when accompanied by mechanical irritation, papillary tumors and adenocarcinoma (Latteri); directly into the intestinal lumen by means of an intestinal fistula, adenomatous and polypoid growths of the mucosa (Genkin and Dmitruk), and insufflated into the bronchi, small adenomas (Kimura). In mice, too, a variety of tumors have been produced. Deelman observed the development of a spindle cell sarcoma in the mouse after applications of tar to the skin. Brancati and Loewenthal produced sarcomas by intraperitoneal injection of solutions of tar in oil; Roussy, Leroux and Peyre, a number of carcinosarcomatous tumors, in 1 case with separate metastases of the 2 tumor forms. By injections of tar Seedorff was able to produce transplantable carcinomas of the breast much more readily in mice than in rabbits—indeed, with the latter animals he was quite unsuccessful. Murphy and Sturm, and Bonne and Stoel, observed in mice the skins of which had been tarred the frequent appearance of adenocarcinomatous nodules in the lungs, the latter along with 1 carcinoma of the palate and 2 of the esophagus; with the direct application of tar to the mouth, Bonne later found that gastric papillomas, which had also been occasionally observed after tarring of the skin, were of more frequent occurrence, as were the oral and esophageal cancers. Schabad, after the intrarectal insertion of tar for 6 months, obtained 1 perianal carcinoma, 2 primary tumors of the lung and 1 papilloma of the fore-stomach; Koose and Cordes likewise observed unduly frequent tumors of the lung and mediastinum in tarred mice, and Schabad in a later report found that there appeared to be a hereditary transmission of this acquired susceptibility to pulmonary tumor, since while they occurred in 27.8 per cent of the tarred mice, as compared with 3.5 per cent of normal animals, in the offspring of the tarred mice they were present with a frequency of 50 per cent.

In view of the decided cancerogenic properties of coal tar, it would seem that the active substance could be isolated and some light thrown on its mode of action by its chemical properties. All efforts in this direction have proved fruitless, and indeed it appears rather probable that there may be in it a number of cancerogenic substances, or that the effect may be due to a number of chemical substances acting together, rather than to one. As a matter of fact, cancerogenic action is not a universal property of coal tar, or at least not one possessed in equal degree by tars from different sources. It has already been mentioned that Fibiger and Bang obtained successful results in one series in 100 per cent of their attempts. In contrast to this stands the work of Mertens, who in 1923 reported a single successful result in 200 attempts



with mice, rats and rabbits. While such differences may depend in part on the source of the tar, in large part they appear to be associated with its mode of production. Even before the study of experimentally induced tumors, Ross in 1918 had summarized the cancerogenic action of various pyrogenous products, as observed clinically, and had stated these as follows:

Obviously cancerogenic: coal tar, pitch (concentrated tar), soot, shale oil and grease. To these Ross added a number of substances of somewhat unrelated character.

Doubtfully cancerogenic: the anthracene fraction of tar distillates and petroleum.

Noncancerogenic: coal itself, blast furnace tar, natural pitches, naphthalene and petrolatum.

The investigation of materials by experimental methods permitted a much more detailed study; in 1921 Bloch and Dreifuss found that the active fraction of their tar distilled at a temperature of over 300 C., and was free from phenols and bases; with this fraction they were able to secure a tumor incidence of 100 per cent. In the following year Murray was able to secure active material after successive extraction of tar with water, alcohol and ether, the last extract giving 50 per cent successes, while the alcoholic extract was not as active. Kennaway, writing in 1923 and 1924, found the active effect manifested by the fractions boiling at higher degrees, between 230 and 500 C. Of tars of various characters, lignite tar, gas works tar, producer gas tar and coke oven tar were apparently cancerogenic, while this effect was not shown by blast furnace tar. Deelman found that the action of tars from horizontal retorts was more pronounced than that of tars from vertical retorts—a matter presumably of relative temperatures, as Kennaway in 1924 reported similar findings for the less heated tars from high retorts. Hoffmann, Schreuss and Zurhelle distilled coal tar into 7 fractions, of which only that boiling at 380 C. showed cancerogenic properties. Jordan found that in the case of a gas works tar of high cancerogenic power, the earlier stages of epithelial hyperplasia developed much more promptly with the residue left after distillation at 400 C. than with the distillate at this temperature. Deelman also found that the pitchy residues of distillation were active in the induction of cancer, and the active material could be extracted from this by toluene, benzene and acetone, leaving an inactive residue. Teutschlaender likewise found that the active material could be extracted from tar by benzene, although the extract was not more potent than the original material. Unlike the general run of the aforementioned results, which would indicate that the agent is present almost wholly in the higher boiling fractions of the tar, Maisin found a fraction boiling at relatively low degrees, from 300 to 350 C., which was actively cancerogenic, and Twort, with Ing

and Fulton, found that the activity was present at times in the higher, at times in the lower, boiling fractions. It could be extracted by methyl sulphite or sulphate, trinitrophenol and ethyl alcohol, and was destroyed or reduced in amount by sulphuric acid, as well as by processes of oxidation or reduction. As a matter of fact, extreme temperatures were found by Kennaway, who heated an active 600 C. tar to 1000 C., to destroy the property; he also found that with a tar obtained from acetylene the portion prepared at a temperature of 700 C. was much more active than that prepared at from 800 to 900 C. On the other hand, isoprene tar prepared by heating to 820 C. was more active than that produced at a temperature of 720 C. With tars produced by heating the same coal to different temperatures, Kennaway found the most active fraction to be that heated to 1250 C., while those heated to 560 and 450 C. showed descending degrees of activity. To the extent that the active principle may be associated with higher boiling fractions, it would appear to be of aromatic character, as Kennaway found that at higher temperatures there was a conversion of aliphatic into carbocyclic compounds. Although it has often been suggested that the cancerogenic action is due to relatively complex combinations—nitrogen-containing according to Philippon, sulphur-containing according to Hammett, while Bayet and Slosse hold that it is due to the presence in the tar of arsenic—there is little evidence to support these views. Fibiger and Bang produced cancers with tars containing the merest vestiges of arsenic; Leitch failed to induce cancers in mice with a Scotch blast furnace tar with an arsenic content equal to that of actively cancerogenic tars from other sources; Mandl and Stöhr also got negative results with an arsenic-containing tar; Möller obtained cancers with a tar without traces of arsenic, and Jordan produced 100 per cent cancers in a limited number of mice with a tar free from arsenic. O'Donovan, too, failed to find arsenic present in British tars of known cancerogenic power, and de Coulon could find no relation between cancerogenic activity and arsenic content. As to nitrogen and sulphur content, Bloch and Widmer found that the action was due apparently to neutral hydrocarbons, entirely free from nitrogen, sulphur and arsenic. Perhaps the most conclusive evidence, however, is that of Kennaway, who with an artificial tar made by heating isoprene—methyl divinyl,  $\text{CH}_2:\text{C}(\text{CH}_3):\text{C}:\text{CH}_2$ —in an atmosphere of hydrogen produced cancers in mice more effectively than with many specimens of coal tar. Apparently the active fraction contains carbon and hydrogen only, except that the possibility of other elements being present in minute amounts as impurities could not be absolutely excluded. Later, the same writer reported the production of a cancerogenic tar by heating acetylene to temperatures of from 700 to 900 C.—an experiment that seems to indicate clearly the hydrocarbon character of the agent. Vaubel sug-

gested that the activity of cancerogenic agents of this character is due to their possession of complexes with a double carbon linkage, by which they have a labile molecular structure, susceptible of activation to ferment-like activity.

A number of substances isolated from coal tar have been studied either singly or together for possible cancerogenic action. Acenaphthene, anthracene, acridine, aniline, benzene, carbazole, chrysene, fluorene, phenanthrene, picene, retene, toluene, truxene and xylene, all representing compounds found in the higher boiling fractions of tar, were studied singly by Kennaway with negative results. Carbazole was later tested by Maisin and DeSmedt, who obtained with it one cancer in the mouse; but as they pointed out, the product used had undergone decomposition by light and could not be regarded as pure. Twort and Fulton observed some slight cancer-producing effect with chrysene alone. Mixtures would appear to be more effective; de Coulon found that carbazole, anthracene, naphthylene and vacuum tar would produce cancers if applied together, but were not effective singly, and Twort and Ing reported the causation of cancer by the application of a mixture of chrysene, truxene and benzerythren, the first two of synthetic production, but with some doubt of the purity of the chrysene.

Much of the uncertainty of the results obtained in the induction of tumors by tarring is due to the long and variable time limits necessary for the successful completion of the experiments. Yamagiwa in his earlier publications stated that in the rabbit the tar had been applied at intervals of 2 or 3 days for from 103 to 565 days before undoubted cancer appeared. In a later publication cancer was reported as occurring as early as the fifty-fifth day, but with a usual period of 150 days or more. Halberstaedter gives 6 weeks as the time necessary for the appearance in the rabbit of epithelial thickening, not necessarily malignant, and von Witzleben 5 weeks for the appearance of tar warts, after applications of tar twice or thrice weekly. Itchikawa and Baum obtained growths more quickly by tarring large areas and carefully removing the crusted tar before applying more; in this way they produced cancers in the rabbit in as few as 47 days, and with over 90 per cent successes by the eightieth day. The first precancerous changes were noted in 36 days, which is not dissimilar from the 30 days stated by Ciechanowski, Morozowa and Wilhelmi as the time necessary for these changes.

In the mouse, the period necessary for the development of precancerous and cancerous changes is not particularly shorter. Tsutsui obtained his cancers after 100 days or over; Fibiger and Bang observed precancerous warts in from 4 to 8 months, with definite cancers after about 5 months in some cases. Lipschütz obtained papillomas in from 88 to 125 days; Bierich, and Bierich and Möller, observed them earlier,

although cancers did not appear until the fourth month, and Murray and Woglom obtained cancers after about 110 days.

Not only is a considerable time required for the appearance of the tar cancers, but the continuation of the tar applications over a long period is also necessary; Bang found that 1 month's painting of mice was entirely ineffective; after 2 months there was about 20 per cent development of cancer, after 3 months about 70 per cent and after 4 months 100 per cent. Teutschlaender in an investigation of cancer in the briquette industry found similar, though of course much longer and less definite, relationships there in the occurrence of the industrial cancers, and recommended less prolonged employment as one element in prophylaxis. Not only did Bang find that cancer was less certain of occurrence after limited periods of the application of tar, but that in those circumstances its onset was delayed, as many as 8 to 10 months being required for its onset after painting for 2 or 3 months. Occasional reports of the exceptionally early development of tar cancers occur, however. Deelman, using an especially active tar, produced cancers in mice in periods of from 3 to 4½ months; Murray observed them as early as the third month, and Foerster, using a tar distilled in a horizontal retort, in 8 weeks. Babes and Serbanesco reported the extraordinarily early development of tar cancer of the rabbit's ear in 2 cases within 18 days. The cancers appeared on the side of the ear opposite to that of the applications, and part of the effect was attributed to the mechanical irritation of scratching.

As regards number of applications, within minimal limits this appears to be almost a constant factor, as Deelman found that with applications at two-day intervals, 22 were required for the appearance of macroscopic skin changes in mice; with three-day intervals, 18; at four-day intervals, 19; at five-day or six-day intervals, 17, and at seven-day intervals, 18. Even when tarring is discontinued before the appearance of macroscopic changes, a process may be initiated that becomes manifest only later. Leitch, with mice painted until the appearance of papillomatous lesions, found that with cessation of the tarring some of the papillomas disappeared immediately or after some continuation of growth; that some grew slowly but did not become malignant, and that some progressed to malignancy just about as they would have had the tar applications been continued. Later he discontinued the painting before the appearance of papillomas, and in 20 mice later observed temporary warts in 4, papillomas in 4 and cancers in 6. The interval between the establishment of potential malignancy and its actual onset is termed by Bang the latent period, and Leitch's work would show that this is obviously longer than the interval required for the appearance of malignancy in previously benign papillomatous lesions. The latter period was found by Bang to be with the mouse from 8 to 9 months,

corresponding proportionately quite closely to the intervals noted to have elapsed between the cessation of occupational irritation and the onset of cancer in man.

The delay in onset of cancer can be reduced if to the almost specific chemical irritation there is added some other factor tending to promote cellular reproduction or division. The experiments of Murphy and Landsteiner, of Carrel and of Laser have been cited, in which malignant growth was induced in fowls by the addition, more or less directly, of tar to embryonic tissues. Not entirely dissimilar were the experiments of Deelman on mice, in which he found that scarification of the skin previous to the applications of tar greatly expedited the appearance of malignant growth, and other experiments by the same worker, in which he was able to localize the site of tar cancer by scarification of areas in the process of tarring. Deelman's observations, which have been confirmed by a considerable number of observers—Teutschlaender, Reding, Rapui, Simoes-Raposo and Cramer—have lacked confirmation by others—Mandl and Stöhr, Truffi, Roussy, Leroux and Peyre, Ludford, Daels, Derom and Parodi, with experiments either identical or along similar lines. Deelman's explanation of his observations is quite other than that suggested here; it is based on the more intimate contact of the tar with the deeper and more actively reproducing layers of the epithelium. As bearing on Deelman's explanation, the procedure suggested by Blumenthal, in which the penetrability of the tar into the skin is enhanced by preliminary cleansing with petroleum-ether, was tried with rabbits by Remond, Sendrail and Boulicard, with negative results; but similar experiments on mice reported by Bartozek, and on rabbits by Bittmann, resulted in early development of cancers—Bartozek's mouse cancers appearing after 68 days, as compared with the 103 days required by controls, and Bittmann's cancers in rabbits appearing after periods as short as from 14 to 36 days. In some of the experiments just referred to, on the relation of injury to tar cancers, the trauma was induced by heat, with which the effects in general appear to be similar to those of mechanical injury, providing the temperature is not too excessive. Derom, for instance, found that with temperatures in the neighborhood of 50 C., the appearance of the tumors was hastened and the incidence was somewhat in excess of that in unburned controls; but with temperatures ranging from 60 to 70 C., and with subsequent applications of tar, the incidence of tumor was diminished.

Heating of the tar itself appears to be particularly conducive to rapid development of tumors. Effects of this sort were observed by Petit, Derom and Lecloux, by Remond, Sendrail and Boulicard and by Findlay, who with a single application of tar or of its chloroform extract, heated to 70 C., caused skin cancer in mice. Analogous clinical

observations were reported by Bang and by Huguenin of patients in whom a skin cancer appeared after a single burn with hot tar.

The experimental investigation of tar cancer includes studies of attempted modification by other factors, both of local and of general character. Yamagiwa, who injected hydrous wool fat along with tar into the breasts of rabbits, observed a lessened effect in these circumstances, although in later experiments he and his associates believed that they found evidence supporting the view that the feeding of hydrous wool fat to tarred animals accelerated the development of malignant growth. Somewhat similar results were reported by Roffo, to the effect that the feeding of cholesterol likewise accelerates the onset of malignant growth. As regards other experiments on the local effects of applied lipoidal material, Lecloux found, as did Yamagiwa with hydrous wool fat, that the application of sodium oleate or of oleic acid, in this case between the periods of tarring, delayed the response to the latter procedure. Berenblum found that the addition to tar of dichlorethyl prevented its effects, a result that might be explained possibly by dilution, were it not for the fact that Hieger showed that some tars, at least, may be diluted ten times without evident diminution of their cancerogenic effect. Testing the action of mustard oil in its relations to tar cancer, Sobolewa found that, if applied before tarring, it causes epithelial atrophy and hinders the cancerogenic effect of the tar; if applied after the cessation of tarring, it hastens the development of tumors.

More general modifications are those of von Witzleben, who in rabbits in which carbohydrate metabolism had been interfered with by the injection of epinephrine and dextrose found exceptionally prompt development of the precancerous changes incident to tarring, while in other animals given injections of insulin the ears were intact after 7 months; von den Borne tried blockage of the reticulo-endothelial system by the injection of trypan blue, and found that this procedure prevented the onset of cancer. Choldin found that in mice the appearance of cancer could be accelerated by preceding repeated injections of indol or of arsenic, and Reding made the curious observation, in a few animals only, that the subcutaneous injection of colloidal magnesium or calcium, or the insertion under the skin of small amounts of metallic magnesium, restrained the appearance of tar cancers, presumably by an effect on the reticulo-endothelial system similar to that observed by von den Borne.

The character of the tissue changes induced by tarring has been a matter of repeated histologic study. There is general agreement that as regards the early epithelial changes, these take the form of gradual, on the whole orderly hyperplasia, either in the form of papillary outgrowth or of delimited downward penetration. Babes, however, did not concede this primary hyperplasia, and regarded the epithelial proliferation, at least in the rabbit, as the consequence of regeneration of tissues

primarily partly destroyed, and Jonkoff expressed the belief that the epithelial overgrowth alternates with intervening periods of atrophy. But according to the more generally confirmed views, there is a progressive continuation of the hyperplasia, with accumulation of excessive keratinized cells, eventually the appearance of atypical proliferation in the basal cells, and finally definitely invasive growth. As to the changes in the corium, there is more diversity of opinion. Early vascular dilatation is the rule, a dilatation that Itchikawa and Baum would ascribe to paralysis of the peripheral nerves; there is some infiltration by eosinophils and lymphocytes, and the elastic fibrils show characteristic changes, which, however, have been variously interpreted. Bierich regarded these as showing an increase, which at first he considered as due to an actual increase in quantity of elastin; but later experiments in conjunction with Rosenbohm, in which they observed similar changes after the immersion of tissues in weak lactic acid, led them to the opinion that the change was merely a swelling rather than an actual increase of fibrils. This view is shared by Philippson and by Sedginidse, while Ulesco-Stroganova regarded the changes as those of actually increased fibrils. The importance of nervous mechanism in the formation of the tar cancers is variously estimated and has been in part a matter of morphologic study. Julius reported that after tarring of the skin in mice there is an excessive growth of subcutaneous nerve fibers, and Itchikawa and Uwatoko believed that they could find a free development of nerve fibers in the cancers themselves. Experiments to determine the functional relationships of the innervation to the tar cancers have given conflicting results. Section of the cervical sympathetic supply to the rabbit's ear, in the hands of Auler, led to marked restraint of the development of the usual hyperplasia, while Itchikawa and Kotzareff obtained directly the reverse result, and observed inhibition, in the way of regression of already induced tumors, after section of the central nerve supply. Repetition of these experiments by Remond, Bernardbeig and Sendrail and by Eiger and Czarnecki gave results that confirmed those of Itchikawa and Kotzareff. Pigalew, too, observed the failure of cancerous changes in the ear of the rabbit after section of the central nerve supply, and he reported that intracranial injections of tar hastened the appearance of skin cancers at the sites of local tarring. Cramer studied the effect of tarring of skin that, by detachment and auto-implantation, was temporarily deprived of its innervation. In these conditions, the tarring caused cancers of these areas, although usually later than in intact skin. His results he interpreted as indicating a lack of effect of nerve control on cancerous growth, but a decided importance of such control in regulating the changes of precancerous inflammation. In general, it may be said that the morphologic study of the changes incident to the development of the tar cancers has thrown

little light on the problem of the origin of cancer; fundamentally, they appear to be essentially those of chronic inflammation, and the gradual onset of undelimited growth shows nothing to betray the mechanism by which it is caused.

As nothing in the nature of a clue to the genesis of cancer has been revealed in the local changes, to what extent may general systemic alteration be concerned in its origin? That prolonged tarring results in such changes is unquestionable. Lipschütz in 1922 and 1923 reported disturbances of melanotic pigmentation after the application of tar to gray or black mice, and inferred from these a general metabolic disturbance. That this action included other effects was indicated by Maisin and DeSmedt in 1924, with the finding that tarred mice as a rule became sterile. Borghi two years later reported finding in both mice and rabbits a rather generalized infiltration of the reticulo-endothelial system by tar, and called attention to widespread degenerative changes of the parenchymatous organs and especially the frequent occurrence of hepatic cirrhosis, with the implication that the former changes at least were of probable importance in the genesis of cancer. But in the same year Döderlein was able to show that the cachexia manifested by many tarred animals had little relation to the onset of cancer, since this might be present to a pronounced degree in animals in which cancer failed to develop. Bonne, a year later, found that the speed of development of cancer bore a relation to the size of the area tarred, even though the region actually becoming cancerous remained small, indicating that the absorption of material from the tar might play a part in the phenomenon. Guldberg in the same year confirmed the presence in tarred animals of the changes reported by Lipschütz and Döderlein; Leitmann and Davidson again called attention to the frequency of cirrhosis of the liver in tarred rabbits, and Fischer-Wasels and Büngeler announced conclusions like those of Döderlein, and believed that there was no connection between remote degenerative changes and local development of cancer. There does not at present appear to be any way of definitely determining the possible relationship of these internal changes to the inception of cancer, but there are rather definite indications that in addition to the local effect, there is a generalized factor in the cancerogenic action of tar. The existence of long latent intervals between the cessation of tarring and the appearance of cancer suggests the evolution of changes more deeply seated than in the exposed tissue itself, as does the fact, as shown by Deelman, that this latent interval is almost independent of the frequency of the tarring. Also the fact shown by the same investigator that in tarred mice, with applications insufficient in themselves to cause cancer, an additional element of trauma could induce malignant growth, points in the same direction. It is true that such evidence is not decisive, since



the latent malignancy could be present in the epithelial cells themselves, but it certainly suggests the possibility of systemic alteration, particularly when it is borne in mind that this effect may be manifested by injured epithelium at the outskirts of the tarred region, where there had been no previous evidence of proliferative change; a purely local effect would imply what Deelman suggested, that there is a latent tendency to abnormal proliferation transmitted by parent epithelial cells to their regenerating offspring. Along somewhat similar lines is the purport of an experiment by Schabad, who found that while there was, as a rule, no recurrence of completely excised tar cancers, the animals still showed a pronounced tendency to the development of new tumors. Finally, there are the instances already cited of the development of tumors, at sites remote from those of the tar applications—as, for instance, the remote appearance of respiratory tumors in mice after tarring of the skin, and the appearance in similar conditions of tumors of the proventricle in the rat. But none of these can be regarded as decisive, since it is impossible to exclude completely the possibility that tar may have been transferred by the activities of the animal, or in the case of the respiratory tumors, by insufflation. That the latter are not due to the inhalation of volatile constituents from the tar has been shown by several experiments—by Nather and Schnitzler, who exposed mice continuously to the fumes of tar for as long as 18 months without observing the appearance of tumors, and by Smith, with similar experiments. The experiments of Fischer-Wasels, in which he applied tar to different areas, and later injured portions of skin to which tar had never been applied and observed the development of cancers at the site of these injuries, while very suggestive, are not entirely free from the same criticism, as is the observation by Kreyberg that in tarred mice cancer may appear in untarred areas after cauterization.

*Other Pyrogenic Agencies.*—Although the coal tar cancers have been the most thoroughly studied of the artificially induced neoplasms, they are only representative members of a large class of tumors that may develop in similar auspices. Mention has been made of the cancers induced by the application of tars made from isoprene and acetylene. A large number of substances of pyrogenous origin have similar effects. Nakamura, in an investigation of the cancerogenic action of a wide variety of tars obtained by the heating of vegetable material, found that pityrol, a tarry substance obtained in the dry distillation of rice bran, had such action. Kennaway found that cholesterol heated to about 800 C. yields a cancerogenic substance, although cholesterol itself has been found by Garschin to cause epithelial hypertrophy. Twort and Fulton were able to secure an unusually active tar by heating pinene to 850 C., and a less effective one from turpentine similarly heated. The list of cancerogenic pyrogenous materials may be considerably

expanded from clinical data. Soot, as the agent in chimney-sweeps' cancer, has, in the hands of Passey, yielded, after alkalization and extraction with ether, a material that produced cancer in 50 per cent of treated mice that survived after three months. Later he and Carter-Braine were able to obtain an active fraction in this material after distillation at 20 mm. pressure at a temperature of over 190 C. Oliver reported the unduly frequent occurrence of skin cancers in asphalt workers and in makers of lubricating grease, this grease consisting of a mixture of coal oil, lime and resin, mixed hot, and gaining contact with the skin in this heated state. Kirk, Davis and Kuntzel published clinical discussions of paraffin oil cancers, long previously described by Volkmann, and Wood observed that this is a matter of the crude product, pure paraffin lacking this effect—though Davis has reported the occasional development of malignant growth in the granulomas caused by the deep injection of presumably pure paraffin. Shale oil, largely used as a lubricant in the cotton-spinning industry of Great Britain, in circumstances that permit the saturation of the anterior portion of the clothing of the operatives, was definitely shown to be the cause of so-called "mule-spinners' cancer" by Leitch, who caused tumors in mice even with rather highly refined oils from this source. Scott found that the shale oils contain persistent sulphur compounds, in which respect they apparently differ from most other cancerogenic substances, and that their harmful effect is enhanced by repeated distillations; Twort and Ing showed that the effect is not altered by the use of the oil as a lubricant, and that the higher boiling fractions are the most active, the shale oils in general comparing with the coal tars in cancerogenic power. Incidentally, the onset of the shale oil cancers is preceded by a period during which the skin is the site of relatively quiescent warty growths, described by Cochrane, similar to those that frequently precede tar cancers in animals and man. Although Norris in 1914 reported that the active material of coal tar was contained in the anthracene fraction, this, as has been seen, has not been substantiated by experimental evidence, and anthracene and the anthracene oils do not appear to be a frequent cause of cancer in human beings, occasional cases only having been reported by Teutschlaender and by O'Donovan, the latter having seen it in elderly workers in impure anthracene. Of other substances of the general class of those discussed here, there may be mentioned pine tar which induced cancer in a case reported by Veiel, and creosote, which in an apparently altogether exceptional case, cited by Cookson, by long-continued irritation produced cancer of the skin of the hand.

Apropos of chimney-sweeps' cancer, the disease no longer appears to be of industrial importance, as in late years it has shown greatly diminished incidence. This was ascribed by Butlin to the use of more

protective clothing and greater care as to personal cleanliness, and by Lawson to the diminished commercial demand for soot; the latter did not attach any importance to the prohibition by law of the use of children as sweeps, owing to the fact that this cancer almost never developed during childhood—fallacious reasoning in light of later knowledge of the latent period of the irritational cancers. Richter, writing of chimney-sweeps' cancer in Germany, accounts for its almost complete disappearance there as due to increased care in regard to cleanliness.

*Scharlach R and the Fat-Soluble Dyes.*—Another group of substances capable of inducing active epithelial proliferation is that of the fat-soluble dyes. In 1906 B. Fischer reported experiments in which he observed, after the subcutaneous injection of scarlet red, extensive overgrowth of the squamous epithelium, which he interpreted as being due to an attractive action of the dye for the epithelium. Although the local changes were almost indistinguishable from those of cancer, they were not indefinitely progressive, although Fischer believed they would become so if the chemotactic action were sufficiently continued. Similar effects were seen with sudan III and with isophenol—all alike in the matter of fat solubility, but as regards isophenol of quite unrelated chemical character. Surface application did not produce this effect. McConnell and Helmholtz in the next year confirmed Fischer's findings, and the latter observed in one instance what appeared to be proliferation of mesothelial elements as well, in the form of numerous free islands of cartilage in the treated ear of a rabbit, apparently formed by metaplasia from fibrous connective tissue. Barratt likewise confirmed Fischer's results, and reported that in the proliferating epithelium there was a frequent occurrence of reduced mitoses, with from 14 to 18 chromosomes as compared with the normal somatic count for the rabbit of from 28 to 36. Snow, on the other hand, observed after the injection of scarlet red at most only the changes of a relatively slight chronic inflammation. Although Fischer's experimental results were in general confirmed, his explanation was not so usually accepted. Jores called attention to the fact that there did not appear to be any evidence that the dyestuff attracted the epithelial growth, nor indeed that it affected this tissue at all unless already in contact with it; he and Wyss and Stahr regarded the element of the mechanical pressure of the injection as an essential factor, which, exerted within the limited volume of the rabbit's ear, either separated the epithelium from its normal contacts and so forced it to obtain nutrition at the expense of the adjacent cells (Wyss), or, acting in addition to the destructive effect of the dye, caused a hyperregeneration (Jores). Although they added little additional light on the causation of cancer, Fischer's experiments

served to introduce the use of these dyes into medicine as stimulants to epithelial repair, as in sluggish ulcers, a use suggested by Krajca and by Schmieden, and obviously not in accord with the importance of mechanical pressure as alleged by Jores, Wyss and Stahr. Werner in 1908 found that the injection of strong solutions of scarlet red would greatly accelerate the growth of inoculated tumors, an effect that did not appear to be chemotactic, but irritative. Practically all the earlier work on scarlet red and related substances was done on rabbits; in the rat, however, Levin found that its effect was outstandingly that of stimulation to connective tissue growth. Stoeber obtained results similar to those of Fischer with a large number of fat-soluble dyes, the most effective of which he found to be naphthylamin; he, too, would dismiss Fischer's chemotactic theory, since in hair follicles he observed that the effect was that of a general concentric hypertrophy; but in his experience direct contact between the dye and the growing epithelium was unnecessary. Meyer regarded the epithelial overgrowth as essentially a chronic inflammatory change occasioned largely by local circulatory changes; it did not take place in the absence of connective tissue overgrowth, nor of the accompanying hyperemia. While Hayward regarded amido-azotoluene as the active ingredient of the scarlet red, White reported that similar hyperplasia could at times result from the injection of oleic or palmitic acids, though not of neutral fats or of fatty acids plus cholesterol. That the epithelial growth incited by irritants of this type is delimited is a matter of rather general agreement—Mori, Haga, Marie and Clunet, Bullock and Rolidenburg, von Lamezan and von Hanseemann all failing to obtain growth of more than limited nature, Haga even after the additional action of cancerous juices, with injections into the mammary gland. Bullock and Rohdenburg, indeed, observed that cessation of proliferation took place even before the complete absorption of the injected material, and ascribed the growth to the action of the irritant in causing a continuous, albeit limited, amount of cellular death, with the ensuing disturbance of equilibrium the essential factor in the determination of the hyperplasia. More recently, however, there have been a number of reported instances of induction of true malignant tumors by the use, usually in combination, of this class of substance. Umehara reported that in the case of a transferable benign tumor of the rat he was able, by the injection of sudan III, to cause it to take on the features of a malignant growth, although these tended to disappear in later generations. Yamagiwa and Ohno produced 3 carcinomas in fowls by the injection from 1 to 5 times, of an oily solution of scarlet red into the wall of the fallopian tube. Eber, Klinge and Wacker found that a diet rich in scarlet red and cholesterol accelerated the development of tar cancers in mice, and that the diet alone could cause in these animals internal changes of

degenerative character, especially hepatic cirrhosis, such as are found in tarred animals. In connection with the administration of arsenic, Fischer-Wasels reported that repeated injections of scarlet red into the breast in rats caused cancers of that organ. Additional negative results are those of Burckhardt, who failed to obtain cancerous growth in autoplasmic transplantation cysts after the injection of scarlet red—similarly negative results were obtained in these circumstances with tar as well—and of Strauch and Bernhardt, who found that even after splenectomy or other interference with the reticulo-endothelial system, scarlet red was without cancerogenic effect.

*Tobacco.*—Akin to the action of the various pyrogenic agents in the excitation of cancerous growth, at least in the sense that the action is largely a local one, is that of tobacco. As has been stated, this effect was first described by Soëmmering in 1795. Knowledge of the subject rests almost exclusively on clinical observation, as experimental investigation has as yet yielded results in no way comparable to those obtained with tar. The only absolutely successful attempt at causation of cancer by tobacco has only very recently been reported by Roffo, who painted the ears of rabbits over long periods with a watery solution of its combustion products, and so produced skin cancer in 1 of 10 animals. Brosch in 1900 tried the effect of applying tobacco juice to the skin, and observed after this atypical, but not cancerous, proliferation. Similar results were obtained in 1910 by Stoeber and Wacker, using pyridine, and in 1912 Wacker and Schminke obtained, with tobacco tar applied to the ear of the rabbit, extensive and atypical epithelial overgrowth, in some cases with actual invasion of the underlying cartilage, but no definite malignancy. In 1923 Hoffmann, Schreus and Zurhelle prepared a tobacco tar as free as possible from nicotine, to avoid the intoxicant effects of this. With this, applied to the skin of the mouse 3 times a week for 80 weeks, there was observed an initial falling out of the hair, with localized hyperkeratosis, which was followed by a temporary reappearance of the hair, but no changes occurred other than such as were of obviously inflammatory character. In 1928 Helwig, using an unmodified tobacco tar also on mice, produced extensive ulcers, which, however, healed when the applications were discontinued; if to the tar there was added a chloroform-ether extract of tobacco, atypical epithelial overgrowth of limited character followed, which later disappeared spontaneously. Some indirect experiments of Freund and Kaminer are also of interest; they found that while normal rat epithelium preserved its destructive action on cancerous epithelium after as long as 3 days' immersion in salt solution, immersion in tobacco juice quickly destroyed this power. On the clinical side, however, the evidence is on the whole direct and convincing, especially,

as concerns carcinoma of the lip. After Soemmering, Rechnitz, Melzer, Stugocki, Bouisson, Thiry and Rapok are among the earlier clinicians who emphasized the importance of tobacco in the causation of these cancers. Additional, albeit indirect, evidence is afforded by the sex distribution of this particular form of cancer, and while the several collections of data naturally show considerable variation, they all emphasize its preponderance in the male sex. Janowsky, Steiner, Wörner, Bencke, Ribera y Sans, Butler and Melzer are in approximate agreement with a relative incidence in the two sexes of about 10:1. Lortet places it at 7.1:1, Bryant at 17:1, Warren at 18:1, and Broders as high as 49:1. Von Haberer observed that in the Puster Valley of the Austrian Alps, where smoking of the pipe is especially common among the women, cancer of the lip is correspondingly more common in this sex. A similar observation has been made in this country by Pettit as regards the greater frequency of this cancer in Negro women. Practically all of these reports emphasize the importance of pipe smoking, a point that is especially brought out by Broders, who, in 573 cases of cancer of the lip, found that while the proportion of the patients who were smokers was approximately the same as that of smokers in the general population—about 80 per cent of the men—the proportion of pipe smokers among the persons with cancer was 78.5 per cent, while among the general population it was only 38 per cent. Kraft found that cancer of the lip is becoming less frequent in the Tyrol with the diminished use of the pipe, and Czerny reported a similar observation. Occasional special cases serve to emphasize the relationship between cancer of the lip and smoking of the pipe. Scherber called attention to the fact that these cancers usually develop at the point where the pipe is habitually held, and von Hanseemann reported a case in which, after operative removal of a cancer of the lip, the patient held his pipe on the opposite side of the mouth, and a second cancer developed there.

But other uses of tobacco are also associated, though somewhat less definitely, with the appearance of cancer. Carcinoma of the tongue in its relation to the tobacco habit has been discussed by Bloodgood, Whitehead, Pannell, Schmidt, Caird, Foerster, Abbe, Kirkes, Coole, Billroth, Eicke, Gorse and Dupuich, Steiner, Schuchardt, Weber, Bottini, Hof, Czermak, Tiedemann, Krönlein, Cortyl, Menetrier, Butlin and Lazarus; and with special reference to the chewing of tobacco, by Rapok, Bottini, Prinzing and Ochsner. The relations of carcinoma of the other oral surfaces to the tobacco habit are the subject of discussions by Spencer, Steiner, Maurange and Faguet, Schuchardt, Abbe, Ribera y Sans, Feldman, Heidrich, von Langenbeck, Schumacher and Birnbaum. An observation by von Esmarch in this connection is of special interest. In certain islands of the North Sea, youths who wished to learn the art of tobacco chewing were accustomed to obtain from the taverns the

unconsumed residue of the pipe smokers, and after retirement to a secluded neighborhood, to chew this, mixed with pipe juices, until they either became adept or too ill to continue. There is in these islands an uncommon frequency of cancer of the posterior oral cavity, which otherwise is very rare, especially in young persons. One may add parenthetically that it is very improbable, in the light of experimental evidence, that one exposure to even so drastic a procedure as this would suffice to cause cancer; but in a person inured to the effects of tobacco in this manner, excessive continued usage might logically be expected.

In a comprehensive and excellent review of the relations of tobacco to cancer, Lickint undertakes to make out a case for the implication of tobacco as a factor in the causation of carcinomas of the upper digestive tract—esophagus and stomach, as well as liver—principally on the grounds of its known physiologic effects. No small part of the evidence takes the form of the greatly unequal sex incidence of these cancers, and Lickint joins with Prout, Lundahl, Percy, Hof and Hoffmann in assuming that such a relationship exists. Necessarily, however, it is much more conjectural than is the relationship of tobacco to oral cancers, and this is true, too, of pulmonary cancers, which Lickint also believes are related to the habitual use of tobacco, concurring in this with a number of clinical observers, Perret, Joannovic, Kanngiesser, Berblinger, Fahr, Hochstetter, Ferenczy and Matoltsy, and Schönherr, all of whom are inclined to ascribe the recent definite increase in these cancers to the growing use of cigarets. To a certain extent this view is supported by the occupational incidence of these tumors—their especial frequency in innkeepers, waiters, etc., as shown by Young, Russell, Brownlee, Collin and Seyfarth, and, with special reference to tobacco dust, the occurrence of pulmonary cancers in cigar and cigaret makers, reported by Seyfarth, Krompecher, Heilmann, Behla, Benda, Brinkmann, Enger, Langbein, Borst, and Jaeger and Kolb.

With the upper respiratory passages the evidence is possibly somewhat more definite, although still indirect. As regards the nose, little effect could be expected with the present methods of tobacco consumption; but Merat, writing in 1826, when snuff was still extensively used, believed that there was an association between that habit and nasal cancer. In the larynx there is again a preponderance of cancers in men, a preponderance that is ascribed at least in part to the use of tobacco by Fürbringer, Gerhardt, Schech, Philippson, Loebisch, Jankau and Young, Russell, Brownlee and Collin.

Lickint is inclined to implicate tobacco also in the causation of cancers of the urinary bladder, again in large part on indirect and physiologic grounds, as well as on the basis of unequal sex incidence.

With cancers of the lip especially, in their relations to tobacco, it must be borne in mind that in addition to the probable specific irritation of certain constituents of the tobacco, other irritational elements must certainly play a part. The fact that cancer of the lip is so predominantly a cancer of pipe smokers would seem certainly to implicate these factors as at least accessory, particularly those of heat and probably of mechanical irritation. Not only is cancer particularly a disease of pipe smokers, but more especially of clay-pipe smokers, as has been pointed out by Hulke and by Cooper. Here the additional irritative factors are especially conspicuous; disregarding the facility which the rather porous stems of these pipes afford for the seepage of pipe juices, they are rather good conductors of heat, and the adhesion of the superficial epithelial layers to the stem affords a constantly repeated source of mechanical irritation. Short-stemmed pipes are blamed by Vulpian and a number of associates, copper-stemmed pipes by Melzer and von Bruns; obviously with both types opportunities would be greater for excessive heating of the epithelium; on the other hand, Rechnitz, who found the disease usually associated with the use of wooden-stemmed pipes, ascribed this to the fact that they permitted a rather free escape of the pipe juices. Additional, though rather conjectural, evidence of the action of the accessory factors is possibly afforded by the usual location of the cancers on the lower lip, although quite probably the chemical irritation would also be more intense here. In the oral cavity, accessory factors play a definite rôle; especially is this true of syphilis, as has been pointed out by Bloodgood, Scherber, Küttner, Villanova, Bejarano, and Fournier and Poirier and, with special reference to pre-cancerous leukoplakia, by Bloch, Ryall, Erb, Kopp and Zeissl.

In view of the almost exclusively clinical character of the evidence, little that is definite can be said of the mode of action of tobacco as a cancerogenic agent. As regards the chemical identity of the agent, Loebisch would ascribe the cancerogenic effect to creosote, which is only conjecturally present in tobacco fumes and is only exceptionally associated with the causation of cancer. Philippon would ascribe the effect to nicotine, in common with the cancer-producing power that he attached in general to the nitrogen-carbon ring compounds. Pyridine was suspected by Stoeber and Wacker. Tobacco cancers have even been explained on the basis of infection, by Jankau, who believed that the fumes produced a local region of lowered resistance. Incidentally, there is in the literature a curious incident of linked occurrence of cancer, reported by MacLeod in reference to the possible infectious etiology of cancer, but equally open to other interpretations, in which an inveterate pipe smoker, who died of gastric cancer, made gifts of used pipes to three friends, all of whom died of cancer in the next few years—of tongue, stomach and colon, respectively.



*Betel*.—Another of what may be termed habit carcinomas is that shown by the betel, or buyo, chewers of the Orient. This habit, of rather widespread distribution particularly in Malaysia, involves the mastication of a quid formed most simply of a bit of the nut of the areca palm, wrapped with slaked lime in leaf of betel, a species of pepper; it has been reported as associated with unduly frequent cancer of the mouth in India and Ceylon by Bashford, in Siam by Mendelson and Ellis, and in the Philippines by Davis. In its inception the added irritation of carious teeth would appear to play an important part. It is true that Leitch questioned the relationship of the habit to cancer, on the ground that the disease is relatively infrequent when the enormous number of betel chewers is considered. However, in Travancore, in southern India, Bentnall found that buccal cancers constituted 34.9 per cent of all cancers, and that these occurred at an age averaging 20 years younger than in Great Britain, a frequency and an incidence that he ascribes to the current habit of betel chewing. And in Davis' experience there appeared to be an unquestionable direct relationship, which was emphasized by the decidedly early age of usual incidence, about 31 years. Which of the several ingredients is primarily or principally responsible is of course uncertain; Davis believed that the lime was principally concerned; but the coca chewers of the Andean highlands, who are stated also to add lime to the coca leaves before chewing, do not show any especial frequency of buccal cancers, if absence of reports to this effect can be regarded as evidence.

*Aniline*.—Another chemical irritant known to be a cause of cancer almost entirely through clinical observation alone, and which differs from those hitherto discussed here in that the cancerogenic effect is remote, is some form or forms of aniline product, and the cancers associated with it are altogether predominantly of occupational character. Rehn in 1895 reported several cases of vesical cancer that had occurred in workers manufacturing fuchsin dyes; he called attention to the urinary suppression and hematuria observed in these workers especially in hot weather, and emphasized the factor of the somewhat specific chronic irritation in the causation of the cancers. The liability of these workers to urinary disturbance had been noted by Grandhomme in 1878, and again by Starck in 1892, but tumors in these circumstances do not appear to have been reported prior to Rehn. Two more cases of tumor of the bladder in like conditions were added by Leichtenstern in 1898, and in 1912 Leuenberger reported that of the cancers of the urinary bladder seen in 50 years at the clinic at Basle University, over one-half had occurred in employees in the aniline industries. Leuenberger regarded the aromatic compounds, especially the hydroxylated aromatic amido compounds, as the responsible agent. Nassauer in

1919 and 1920 found that the cancers were not entirely restricted to employees, but that they were also found among persons residing near the chemical plants, and from this deduced that the inhalation of fumes might suffice for their production. In his opinion aniline itself was responsible. Several studies of these cancers appeared in 1920. Schwerin was able to report successive stages in their development—both acuminate and villous papillary tumors, warty excrescences, sessile carcinomas and generalized papillomatosis of the bladder, which in their diversity and long course are reminiscent of the experimental tar cancers, as is the long latent period to which Schwerin called attention. Oppenheimer in a study of 20 of these tumors believed that they could be traced to a number of chemical substances—benzidine, aniline and various aniline dyes. In his cases there was a latent period from the time of first employment to that of first symptoms varying from  $9\frac{1}{2}$  to 28 years. He could find no relation between the type of tumor and the particular agent apparently responsible. As concerns benzidine, one of the substances implicated by Oppenheimer, Nassauer, who had not observed cancer in employees working on pure pulverized benzidine, had been inclined to dismiss this from consideration as a causative agent. Jaffe attempted to study the production of these cancers experimentally by exposing mice, rats and rabbits over periods of from 6 months to 1 year and more to the vapors of aniline, toluidine and naphthylamine, but was quite unsuccessful in the induction of tumors of the bladder in these animals.

In a rather recent article, however, Schär stated that in rabbits exposed to the inhalation of fumes of naphthylamine there were found in the bladders of the surviving animals, after a year, epithelial metaplasia, increase and round cell infiltration of the connective tissue and in two animals, papillary tumors of the bladder, one of which was evidently malignant. Engel, approaching the problem from the point of view of excretion, found that betanaphthol appeared in the urine of dogs as sulphates or glucuronates of the amidonaphthols, and similar substances were demonstrated in the urine of human beings, especially in that of employees exposed to inhalation of the dust of the aniline materials, by Kuchenbecker, although they were present only in extremely minute quantities. In experiments in which he fed various amido compounds to laboratory animals, he found that as a rule these were eliminated as harmless sulphates, except in the case of  $\alpha$ -naphthylamine and  $\beta$ -naphthylamine. Hamilton offered the suggestion that the cancers of the bladder originating in the aniline industries may be ascribed to the presence of arsenical impurities. As against this view is the very considerable evidence that cancers of arsenical origin have never been observed in the urinary tract and are almost exclusively found in the skin.

As pertaining to the long latent period, that this is not occasioned entirely by the necessity for prolonged exposure has been indicated by Posner, who reported cases in which the industrial contact had ceased years before the first appearance of a tumor.

*Arsenic.*—Unlike the cancerogenic irritants hitherto considered, arsenic appears to be responsible not so much for the development of cancers locally, as for a generalized effect in which the appearance of cancer is associated with the additional action of other factors. What appears to be the first reference to this action of arsenic is that of Lambe, who in 1809 expressed the belief that arsenic in potable water was the cause of malignant disease. In 1820 appeared the statement by Paris already cited; the first definitely authentic evidence of the relationship of arsenic to cancer appeared in the report by Hutchinson, in 1887, in which he cited the development in several persons, after the long ingestion of arsenic, usually for psoriasis, of cornlike lesions on the palms or soles, which later became cancerous. A considerable number of similar instances have been reported, one indeed, that of White, preceding the appearance of Hutchinson's report, but lacking the suggested association of the arsenical medication with the later cancer; after Hutchinson, cases were reported by Ullmann, Crocker and Pernet, Darier, Lane, Hartzell, Bland-Sutton, Schamberg and Wile. Nutt, Beattie and Pye in 1913 were able to tabulate 30 cases of this sort, and since then additions have been made by Semon and Aliferis. Practically all cases resulted from arsenical medication, principally for psoriasis, but a significant feature repeatedly mentioned in the reports is that the cancers usually develop on the hands or feet, and very seldom at the site of a psoriatic lesion; or they may be multiple, as in the case of Lane, and even then seldom show relationship to such a lesion. In general, the period of the ingestion of arsenic was long, and may have ceased a considerable time before the appearance of the cancer; in the case reported by Crocker and Pernet, none had been taken for 38 years, in that of Wile, for 8 years, and in that of Semon, for 13 years. Another significant feature is the relatively early age at which these cancers may appear; in the series collected by Nutt and Beattie, 25 per cent of the patients were not over 35 years of age. Differing from the foregoing cases in mode of ingestion are the cases reported by Geyer, in which this was either by means of arsenic-impregnated water or by the inhalation of dust from neighboring arsenic mines. To this latter category possibly belong also the frequent cancers of the lung seen in the miners of the Schneeberg district, as described by Rostoski, Saupe and Schmorl; but the ores there, in addition to a high arsenic content, contain a wide variety of minerals—copper, iron, silver, cobalt and other metals—so that the evidence here is not

as definite as in the case of medicinally administered arsenic. Although in the case of the skin cancers following ingested arsenic the action of the latter, as has been stated, appears to be remote and of a rather general character, evidence is not lacking that with suitable local application the effect may be manifested at the point of application. O'Donovan reported a case of skin cancer in such circumstances, following the constant (occupational) irritation by arsenic dust; the Schneeberg cancers as described by Rostoski and his colleagues, to the extent that their etiology appears to be associated with arsenical dusts, appear to be other examples, as do those of the Joachimsthal mines reported by Löwy, although in both radioactivity may play a part. With the Joachimsthal lung cancers, indeed, Sikl, who found no less than 8 of these tumors in the 15 miners who had died during the period covered by his report, regarded radiant emanative material as the probably responsible agent. On the experimental side, Simoes-Raposo reported that by applications of an ointment containing arsenious oxide to the ears of rabbits, he produced cutaneous lesions ranging from hyperplasia to cancrioid tumors. The relations of arsenic to experimental cancer have been the subject of a considerable amount of study. In 1915 Funk studied the effects of the administration of arsenic on the course of the inoculated tumors of fowls, without finding any definite evidence of action; a similar experiment by Schiller in 1926 likewise yielded essentially negative results. In 1922 Leitch and Kennaway fed arsenic trioxide to rats and mice without observing the development of malignant tumors; but the death rate was high from intoxication. Ciechanowski reported that the appearance of papillary and other precancerous changes in the skin of tarred rabbits could be accelerated by the administration of arsenic to them, and Askanazy in 1927 reported that by repeatedly injecting dilute solutions of arsenious acid into transplanted rat embryonic tissues, malignant growth of these occasionally was obtained; in adult rats in the gastric walls of which there had been implanted fetal visceral tissues, the feeding of arsenic was found to induce tumor growth. The experiments of Carrel and of Fischer, who induced malignant growth of embryonic tissue of fowls, the one after implantation into the adult animal, the other *in vitro*, by means of arsenic, have been cited. Petroff and Krotkina<sup>1</sup> reported successful causation of transferable sarcomas in the rat by intraperitoneal injection of suspensions of rat embryo cells along with solutions of arsenic or of indol, followed by repeated administrations of arsenic or of indol. However, a considerable number of more or less close repetitions of the work of Carrel have led only to negative results. Leitch with a similar experiment on 40 fowls, Kauffmann in 30 attempts, Collier and Hartnack with a similar

experiment conducted with mice, and McJunkin and Cikrit with rats have all been unsuccessful, except in the case of 1 animal in the hands of the last-named experimenters. Fischer-Wasels found that in experimentally arsenicized mice local mechanical injury was insufficient to produce tumor, but that if scarlet red was injected into the breasts of female mice treated with arsenic, carcinoma developed in 5 of 18 surviving animals. The effect of arsenic on tumor metabolism was investigated by Dresel, who found that while weak solutions of arsenious acid inhibited the respiration of the Jensen rat sarcoma with some rapidity, the fermentative activity persisted considerably longer, being reduced, however, to about 50 per cent of its normal level after 3 hours.

Although many of the experimental studies of arsenic have indicated its ability to act as a local cancerogenic agent, its outstanding clinical significance, as stated, appears to be as an agent that seems to cause a decided increase of predisposition to cancer, so that under its influence cancers may appear in the human being at an unusually early age, and in sites that are commonly spared, but in which an added element of irritation that in ordinary conditions would be inadequate comes into operation. The only evidence of the nature of this increased predisposition is furnished in the account by Fischer-Wasels of the experiments of Büngeler, who observed in the tissues of rats to which arsenic had been administered in small doses over long periods a distinct but slight reduction of oxidative activity, an increase of anaerobic glycolysis and the appearance of aerobic glycolysis. Similar effects were observed after the application of tar over considerable periods, and with both agents the effect was most pronounced in the skin, although apparent elsewhere as well.

*Indol.*—The action of indol as a cancerogenic agent in many ways parallels that of arsenic, particularly as regards the usually general nature of its action. Aside from the possible association of cancer with intestinal stasis—an association that is more conjectural than proved—present knowledge of this effect is based practically entirely on experiment. Stoeber and Wacker, in a study published in 1910 on the effect on tissue proliferation of products of protein cleavage and putrefaction, observed atypical epithelial overgrowth after the injection into the rabbit's ear of pyridine, indol and skatol, the growths obtained with the two latter substances being morphologically indistinguishable from human epithelioma. Greischer in 1912 found that the effect depended largely on the length of the contact, and could be affected by associated hyperemia, as after section of the sympathetic nerve the arterial hyperemia reduced the degree of hyperplasia, while venous hyperemia after ligation enhanced the amount of growth. Fischl, who

repeated Stoeber and Wacker's experiments in 1925, observed no decided changes, nor was any effect manifested after repeated applications, over a period of 6 months, of an indol ointment to the skin of mice. The experiments of Carrel, in which indol and embryonic tissue injected together into fowls developed into sarcoma of the Rous type, and of Petroff and of Krotkina, just cited, are the most definite indications of its cancerogenic effect. In some recently published experiments, Choldin believed that he was able to show that small doses of indol or of arsenic trioxide increase the susceptibility of mice to tar cancer.

*Occasional Chemical Irritants.*—In addition to the chemical irritants that appear to have a specific cancerogenic effect, there is a wide range of agents that appear to have caused cancer as an occasional, rare and usually late sequel. Bland-Sutton reported a case of skin cancer originating in the site of a burn caused by sodium hydroxide, and Narat in 1925 observed the occasional appearance of growths morphologically indistinguishable from skin cancers after the long application of potassium hydroxide or hydrochloric acid to the skin of mice. Findlay cited a case of conjunctival cancer after a splash of carbolic acid, Mishell one of skin cancer 7 months after a slight sulphuric acid burn, Berner one of multiple carcinomas of the esophagus and stomach 4 months after the swallowing of strong ammonia and Kliment, one of cancer of the esophagus in an inveterate sulphur eater. The etiologic relationship of alcohol to cancer, which has been alleged principally on the evidence of the histories of victims of cancer of the alimentary tract, received apparent confirmation by Krebs, who reported the development of carcinoma in the colon of 2 of 16 mice given repeated intrarectal injections of 50 per cent alcohol. Michalowsky reported that teratomas could be caused in the cock by the injection of zinc chlorate solution, although there is no evidence that these tumors were malignant. In connection with the diverse character of these agents, it is of some interest that Rosenstein could observe no evidence of the induction of tumor after the long-continued application of iodine to the skin of mice. Considering the only occasional occurrence of relationships such as have been just cited, it would appear that the effect of the agent in these cases was simply the induction of an inflammatory or regenerative hyperplasia which, owing presumably to an element of individual predisposition, became unlimited.

#### PHYSICAL IRRITATION

*Mechanical Injury.*—Although the part played by chemical irritants as agents in the production of cancer may be regarded as firmly established by both clinical and experimental evidence, the rôle of physical

trauma in this connection is, for rather obvious reasons, in general more difficult to determine. This is particularly true of mechanical trauma, because of the usual possibility of the action of associated factors. So, for instance, the frequent relationship of cancer of the penis to phimosis, to which Hey called attention in 1823; the frequent occurrence of skin cancers in the scars left by flogging on the backs of sailors, reported by Hawkins in 1835; cancers developed at the sites of old burns, described by von Bergmann in 1873, all suggest such an association, but do not exclude the action of other influences as well. The same is true of the very definite relationship between gallstones and carcinoma of the gallbladder, repeatedly discussed in the literature, and recently, and at some length, by Luelsdorf. Even the experimental work along this line, in which Bullock and Rohdenburg, Kazama, and Maisin and Picard reported the development of neoplasms, either benign or malignant, after the introduction of solid material into the gallbladders or urinary bladders of laboratory animals, leaves open the possibility of chemical or bacterial irritation even in those instances in which the element of chemical irritation was not intentionally introduced. Other clinical instances of the not unquestionable effect of mechanical irritation in the induction of cancer are those of the friction on the oral mucosa of diseased teeth, to which Morris called attention in 1882; the cases cited by Schwarz of the development of tumor in phalanges chronically subjected to mechanical irritation, in one case by an embedded thorn, in another by repeated pricking by a tailor's shears, and of carcinoma of the cervix in which an ill fitting pessary had been the cause of prolonged mechanical irritation, as in the case cited by Dyas. In none of these can it be proved that mechanical irritation was the sole factor. The same holds true for the more or less experimentally observed development of epithelial hyperplasia, in some cases cancerous, observed on the tongues of rats as a result of the embedding there of oat hairs, reported by Stahr and by Secher. At least one case reported by Zahn, in which he observed the appearance of a primary carcinoma of the liver at a site irritated by the friction of a deformed rib, would come more nearly to meeting the probability of purely mechanical injury of chronic character as the inductive agent of cancer; his second case, in which a similar cancer was apparently caused by the mechanical effect of dense adhesions between liver and colon, does so to a less degree. A case reported by Stahr, of epithelioma of the thumb of a shoemaker's apprentice, appearing at the spot where it was repeatedly pricked by his needle, at the early age of 17, appears to rest on an almost purely traumatic basis. Of rather uncertain character are the cases of Henson, in which natives of Natal showed a frequent incidence of malignant melanotic tumors of the foot.

which Henson associated with the deep cracks in the skin of the soles during the dry season.

With single, acute mechanical injury, the probable accessory action by other factors is of course less, but cannot always be excluded. Numerous instances are reported in the literature of the development of sarcomatous tumors in these circumstances. Londner in 1885 reported a spindle cell sarcoma of the breast that appeared immediately after such an injury; Löwenthal in 1895 was able to collect 137 cases of malignant tumor of the breast in similar circumstances; Lengnick in 1899 reported 13 sarcomas and 2 carcinomas that appeared to have been caused by a single injury; Löwenstein in 1906, in a review of a large number of cases, found the element of acute trauma present in 40 per cent of sarcomas; Orth in 1907 reported 2 cases; Rapok found traumatic antecedents in approximately 10 per cent of sarcomas; Coley, in 23 per cent. A curious form of precancerous trauma, among the curiosities in sarcoma causation, was that reported by Ott, in 1910; he found that a sarcoma followed directly on the bite of a dog, and he was able to collect records of 4 similar cases. Maude Slye observed, in 87 mice dying of spontaneous sarcomas, that 11 of the sarcomas were at sites of injury; in these mice there was a distinctly hereditary element. The relationship is much less frequent in the case of the carcinomas, though even with these tumors acute trauma appears to play a causative part occasionally. Ziegler in 1895 stated that single trauma apparently played a causative rôle in 35 of 328 cases of carcinoma. Coley in 1911 reported that he had been able to establish a relationship to single acute injury in 37 cases diagnosed cancer of the breast. Especially interesting in this connection are the isolated cases in which the relationship has been worked out in some detail: that reported by Schöppler, of a carcinoma of the breast apparently caused directly by the impact of a fall; that of Gerdes and Susewind, in which there was the rapid development of a branchiogenic carcinoma following acute injury, and the 2 cases reported by Schad, who observed that tumors with these antecedents are especially frequent in young persons, indicating the likelihood of a precancerous disposition. As a matter of fact, it is practically necessary to consider each case of alleged carcinoma of traumatic origin on its own merits. The relationship is of course one of great medicolegal importance, and certain criteria must be absolutely established to warrant the presumption of causal relationship in these circumstances. Lubarsch especially emphasized as such criteria: (*a*) proof of the injury, (*b*) the causation by the injury of immediately perceptible local effects and (*c*) a reasonable relationship of time between the injury and the appearance of the tumor. Löwenstein added certain postulates that, while not entirely essential, are of



great significance in establishing the association: the immediate recognition of the severity of the traumatic lesion, with a sequel of still greater disturbance; an unbroken sequence of symptoms between the injury and the cancer; the presence of residues of the injured tissues in the cancer, and, if occurring at an early age, the absence of other causative factors such as possible heredity—although, obviously, rigid insistence on this last postulate would introduce injustice. He recognized, however, that each case must be considered with full regard to both clinical and pathologic features. Reported instances of the development of malignant tumors with traumatic antecedents in animals are not common. In addition to those of Slye that have been cited, Hewlett reported the frequent occurrence of horn-core cancer in Indian cattle, apparently caused by injury to that organ incident to their use as draft animals; Beatti reported a carcinoma of the ear of a sheep, evidently caused by an impacted thorn. Attempts to induce malignant tumors experimentally by mechanical trauma alone have on the whole been unsuccessful, with the exception of the vesical cancers hitherto referred to, and the oat-hair cancers in rats. Löwenstein attempted to cause cancers in experimental animals by means of traumatic displacement of epithelium, with complete lack of success. Even the sustained mechanical irritation of implanted kieselguhr, as has been seen, induces malignant hyperplasia so seldom as to exclude this from the practical methods of producing tumors.

*Thermal Injury.*—Thermal irritation, on the other hand, appears to bear a definite and unquestionable relation to the induction of tumors. The increased activity of heated tar in this connection has been mentioned. A number of cases have been recorded in which skin cancers can be traced quite definitely to single burns, though of course with many of these there is the added factor of known chemical irritation. The kangri burns seen in Kashmir, which develop in the scrotal region as a result of the use of charcoal fire pots held between the thighs and beneath the clothing, to which Maxwell first called attention in 1879, and which were reported in considerably more detail by Neve in 1910 and 1911, come in this category; here, in addition to the evident element of thermal irritation, there is a possible added action by substances of pyrogenic origin, as has been suggested by Vaughan. Stahr reported the appearance, in a fireman, of a squamous cell carcinoma of the skin on the forearm where it was repeatedly exposed to the heat of the furnace; while there is a possibility of associated chemical action in this case, it is slight, in view of the known lack of cancerogenic action by coal. Pickerell described several cancers that were traced more or less directly to burns—a rodent ulcer that appeared within 5 months of, and at the site of, a cinder burn; one that appeared

2 years after a cigaret burn, and with more doubtful relationships, a squamous cell carcinoma originating at the site of a burn of the lip incurred 15 years previously. Hauff reported a case of epithelioma of the face that appeared after a single severe exposure to heat and soot, but with an absence of actual burning of the skin. On the experimental side, the adjuvant action of burns in the induction of tar cancers has already been cited, including the production of cancers in tarred animals at sites not used for applications of tar. As concerns thermal change alone, Fuerst in 1898 found that repeated applications of mild degrees of heat or cold to the skin of animals caused epidermal thickening, sometimes eightfold; but apparently nothing was seen that could be interpreted more definitely as tumor growth. Werner observed similar results after repeatedly freezing the skin of the rabbit's ear with an ether spray. In a large series of mice that were subjected to repeated burning of the same areas of skin, Burckhardt and Müller were unable to observe production of tumors, but as they were equally unsuccessful with the x-rays, their methods are not entirely beyond possible criticism. Berenblum reported that in mice there occasionally develop cancers at points on the skin subjected to repeated slight freezing with carbon dioxide snow.

*Injury by Radiant Energy.*—X-Rays: Radiant energy of the shorter wavelengths appears to exert a much more definitely cancerogenic action. Soon after the discovery of the x-rays, their effect in causing a chronic dermatitis, frequently of hypertrophic character, was noted. Mühsam, and Baermann and Linser, described lesions of this sort in 1904, but apparently at that time cancerous sequelae had not as yet been observed. These appear to have been first reported by Wyss in 1906, who even at that time called attention to the greater probability of such lesions developing if some condition of chronic change, such as lupus, had previously been present. He believed that the cancerous growth could be referred to nutritive alterations incident to the arteriosclerosis that is a prominent morphologic feature of the process. Porter and White a year later made a detailed study of the various stages of roentgen dermatitis and cancer, again calling attention to the obliterative endarteritis, and Schümann in that year noted that after undue roentgen treatment for lupus, the sequel might at times be carcinoma of the skin and at times sarcoma of the underlying tissue, an observation that was confirmed by Lindermann. In 1908 Rowntree published a very detailed account of the histologic changes caused by this agent, finding them in their earlier stages to be those of epithelial hypertrophy, followed by atrophy, which was associated with increase of the connective tissue of the corium, and ultimate denudation of the epithelium. The elastic tissues showed early superficial diminution, with

eventual complete disappearance here, but with an increase in the deeper tissues. In the accessory cutaneous structures, hair follicles and sebaceous glands, there were changes similar to those of the skin proper. Final unrestrained epithelial hypertrophy occurred late, but, in general, hypertrophy appeared to be caused by exposures of moderate severity only, heavier doses having an effect predominantly destructive. With the former type of dose, detachment of masses of epithelial cells was observed, and the writer ascribed the origin of the cancers to the stimulation of epithelial growth into a substratum of connective tissue that no longer offered normal restraint to epithelial invasion. He, too, called attention to the frequent occurrence of an obliterative endarteritis. Wohlbach in 1909 observed a slow augmentation of the power of growth on the part of the epithelium, accompanied by a progressive loss of differentiation, conditions that ultimately endowed the cells with the capacity for parasitic growth. In 1910 appeared the first reported instance of intentionally induced malignant growth by Marie, Clunet and Raulot-Lapoints—by the exposure of a rat to heavy, ulcerative doses of the x-rays. It is interesting, in connection with the difficulty of inducing carcinomas of the skin in this animal by tarring, to note that this tumor was sarcomatous. A second sarcoma similarly induced was reported by the same experimenters in 1912. Carcinoma has been induced experimentally by this agent in the rabbit by Bloch, and in the mouse by Jonkhoff; in the guinea-pig sarcoma has been produced by Goebel and Gerard, although in only 1 of 20 treated animals. In a study of the effect of the x-rays on metabolic processes, Frick and Posener found that it restrains glycolysis in part, but also reduces the restriction of oxidation on that process.

Radium: Closely akin to the effects of the x-rays are those of radium, a similarity that was noted by Werner in 1905, who believed that the effects of both were due to their action on lecithin, making this more susceptible to cleavage by ferments. Experimentally, the induction of sarcomas in rats and mice by embedding under their skin tubes containing minute quantities of this material was reported by Daels, who also observed 1 carcinoma of the skin develop after this procedure, and in the breast of a mouse the appearance of a mammary adenocarcinoma after the insertion there of radioactive material embedded in a mixture of paraffin and kieselguhr with a trace of arsenious oxide—truly a shotgun mixture. Schürch and Uehlinger, one and a half years after radium had been embedded subperiosteally in the jaw of a rabbit for twenty days, observed the development of an osteogenic sarcoma at that point. Maisin injected ionium, a radioactive material that emits alpha rays only, and found that this not only hastened the induction of tar cancers, but in fowls, if injected intra-

venously after the implantation of embryonic tissue, greatly increased the growth of the latter, frequently to the point of sarcomatous development.

**Ultraviolet Rays:** Ultraviolet rays, from the quartz mercury-vapor lamp, have been studied to some extent experimentally as to possible cancerogenic effects. Findlay found that long exposure of mice, and more recently, of rats, to these rays caused in them papillomatous or epitheliomatous overgrowth of the skin, and that this irradiation hastened the action of known cancer-producing agents. Rothmann and Barnhardt, on the other hand, found that application of ultraviolet rays to the skin of the rabbit's ear caused only epithelial atrophy; Kohn-Speyer was able to observe no effect of the irradiation on the development of tar cancers in mice; but in the rat, Putschar and Holtz have recently reported the induction of skin cancers after prolonged exposure to ultraviolet rays. Simon reported the development, in an old operative scar that had remained unchanged for 7 years, of a spindle cell sarcoma 1 year after quartz lamp irradiation.

**Sunlight:** From clinical evidence, there would seem to be definite cancerogenic action on the part of sunlight. In 1907 Dubreuil reported that of 162 patients with skin cancer, mainly rodent ulcer, 101 gave histories of outdoor occupations, and that the cancers were located almost exclusively on areas of exposed skin; also that they were more frequent in lightly pigmented persons. Lawrence in 1928 associated the prevalence of hyperkeratoses, rodent ulcers and epitheliomas in Australia with the excessive sunlight, and predicted that similar relations would be found in the more arid portions of the United States—a prediction that I am informed is correct, although I know of no publication conveying the facts. Unna described a condition that he termed "Seemannshaut," of hyperkeratosis and especial predisposition to skin cancer, shown most frequently, as the name would indicate, by aged seamen, but not uncommon in persons who have lived long exposed to the sun. A similar condition is at times idiopathic, and Halberstaedter pointed out that in so-called xeroderma pigmentosum there is the same sensitivity to sunlight as in "Seemannshaut," but in this case is present congenitally.

**Mitogenic Radiation:** Lazarus-Barlow found that occasionally malignant tumors possessed the power of causing electroscopic discharge, and suggested that this might imply the presence in them of radium, and that radium emanations might be the cause of cancer to the exclusion of all others. In this connection, the work of Gurwitsch is of interest in that he found regenerating tissues to be an active source of what he terms "mitogenetic radiation," which, while not of sufficient strength to affect a photosensitive plate, has the power of con-

siderably accelerating nuclear division in growing tissues exposed to it. From the work of Kisliak-Statkewitsch the radiation appears to be produced by ferment activity, as in winter potato buds he found a pro-ferment which, after activation accomplished by wounding of the leptome bundle, generated the radiation. The same writer would ascribe the source of energy in part to autolysis of dead tissues, since the radiation is emitted by dead, but not by healthy, portions of tumors, unless the latter are in contact with dextrose, so that he postulates for them a double origin, by glycolysis by living, and by proteolysis by dead, cells. In either case the phenomenon appears to be due to oxidation, as Gurwitsch and Sorin both reported that it occurs when blood is exposed to air, even after hemolysis. Frank and Salkind found that in the sea-urchin's egg mitogenic radiation precedes the first recognizable evidence of mitosis as a sudden flare-up following a temporary period of increased consumption of oxygen. As Gurwitsch pointed out, the effect is enhanced by the induction of mitosis, with additional radiation, in adjacent cells. Confirmation of the effect of these rays is afforded by the work of Guillery, who was able to demonstrate that in cultures adjacent tissues could affect each other's rates of growth, even though they were physically separated by gaps in the medium, but that this effect was absent if glass strips intervened; he reported even that the effect could be reflected by metallic strips, and so could be explained only by the emission of radiant energy from the growing tissues. On the other hand, Ypsilanti and Paltauf were quite unable to detect mitogenic radiation from mouse carcinoma and Rous sarcoma tissue by the effect on Liesegang rings, although this method was effective with onion roots. To what extent these radiations may play a part in the phenomena of tumor causation it is of course quite impossible to say with any assurance. Since they seem to be common to growing tissues of any sort, it would seem that in tumors they would not be, except possibly quantitatively, of special significance, and that they are to be regarded as a manifestation of the peculiar metabolism of dividing cells. Magrow, indeed, reported experiments that tend to show that the peculiar action of the tumefacient bacteria is due to their emission of radiations of this sort. As to their physical properties, Frank and Gurwitsch reported that a band of ultraviolet radiations ranging from 2,370 to 1,930 angstroms possesses similar effects.

Finally, radiant energy has served as a background for two really extraordinary theories of the causation of cancer—that of Joly, who would ascribe the alleged increase in the disease to diminution in the amount of Millikan's "cosmic rays," and that of von Pohl, who expressed the belief that cancer is due to certain electronegative radiations, which are especially abundant over certain subterranean con-

ducting zones, and who alleges his ability to locate by physical means the houses, rooms and even beds in which cancer has occurred. There would be little profit in discussing either hypothesis.

#### INFECTIOUS IRRITATION

*Syphilis*.—Although infection and cancer do not show the great frequency of association that characterizes many other forms of irritation and cancer, there are enough cases of the latter disease occurring as sequels of infection to establish this definitely as a precursor of cancer. Of the infections most frequently implicated, syphilis and tuberculosis are the most usually concerned, with the former predominant, particularly if lingual leukoplakia be regarded as a lesion of that disease. In 1862 Neligan described leukoplakia of the tongue, and in a specific case predicted the probability of later cancerous change, a prediction that was later verified. In the years following, cases of cancerous development from leukoplakia of the tongue were reported by Clarke, Mauriac, Nedopil, Morris and Weir, the last of whom was able to collect records of 68 cases, of which 31 resulted in cancer. Eve in 1881 cited a number of cases showing more general relationships between chronic irritation and cancer, including among them cases of leukoplakia, chimney-sweeps' cancer and cancer in irritated corns and calluses. While there is the possibility that leukoplakia of the tongue may be of other than syphilitic origin, since in the lip it is a not infrequent sequel of smoking, the usual consensus of these earlier observers was that in most of their cases there had been syphilitic antecedents. As showing more definitely the relationship of syphilis to malignant growth, in 1912 Rohrbach described a case of atypical epithelial overgrowth of the skin in a gummatous syphilid, which was very suggestive of carcinoma, but as it disappeared under antisiphilitic medication, it can scarcely be regarded as having been cancerous. In 1913 Ledermann reported precedent syphilis in 4 cases of laryngeal cancer, as well as cases of oral cancer on a syphilitic basis. Winfield also in that year reported the development of a squamous cell carcinoma in a nodular syphilid, and Whitehouse one of a similar cancer of the palate at the site of a gumma. Nagy described sarcomatous development in a tertiary syphilitic lesion of the uterus. Barinbaum, in a discussion of the relations between syphilis and cancer, concluded that while the actual cases of reported association are not numerous, the matter of causal relationship is scarcely open to question. Here, as is so frequently the case with other forms of chronic irritation, association of other factors appears to play a prominent part. Pettit, in discussing cancer of the lip in Negro women in the District of Columbia, emphasized the importance of an association between syphilis and pipe smoking. and

Schleicher that of leukoplakia, tobacco and syphilis as precursory factors in oral cancer, although he was inclined to doubt the importance of syphilis as the cause of the first condition. A relationship of malignant growth to syphilis has been observed several times in experimental animals; Brown and Pearce described in great detail a transferable carcinoma apparently of the root sheaths of the hair in a rabbit inoculated with syphilis at the site of the cancer 4 years previous to its development, and a later case of melanosarcoma in the eye of the rabbit inoculated at that point with syphilis. Von Niessen has reported that in a rabbit inoculated with syphilis death occurred after 4 years from adenocarcinoma of the liver, and that in 2 mice inoculated, respectively, with *Gonococcus* and *Spirochaeta pallidum*, and with vaccinia, adenocarcinomas appeared over a year later. All of von Niessen's cases lack the definite relationships of localization shown by those of Pearce and Brown and scarcely support his contention that venereal infection, recent or ancestral, is an outstanding factor in the etiology of the tumor.

*Tuberculosis.*—Tuberculosis, especially of the skin, has likewise frequently been observed clinically to be antecedent to carcinoma. Apparently the relationship was first noted by Devergie in 1857. Lang in 1874 reported a case of lupus that developed into carcinoma and cited a number of similar cases. Von Langenbeck in 1875 reported 3 such cases, and attention has been called to the association by Kaposi, Raymond (with 15 cases), Richter, Ribbert and Pautrier, the latter with a case of lupus erythematosus. A discussion, of rather comprehensive character, along with a bibliography, has been furnished by Bastedo. The relationship is not limited to skin cancer. Tauffer described the development of a spindle cell sarcoma in an old lupus lesion; Beck, 3 similar cases, although in these there was the associated element of irradiation. Watsuji observed several cases of cornifying cancer of the lung or bronchus appearing at sites of epithelial metaplasia most usually induced by tuberculosis; Schwalbe, a bronchial cancer originating in a tuberculous cavity. Hamperl reported no less than 6 cases of gastric or intestinal cancer occurring in connection with tuberculous lesions, and Wolf recently described a polypoid carcinoma of the stomach, apparently originating in a tuberculous ulcer. Experimentally there is not the same supporting evidence of the relationship as obtains in the case of syphilis, a fact that may be plausibly explained by the more pronounced lethal action of tuberculous infection in animals, though Jensen has seen the development of 2 transferable sarcomas in rats inoculated only 2 months previously with acid-fast bacilli derived from a pseudotuberculous bovine enteritis. The relations of tuberculosis to cancer are complicated by the apparent antagonism between the two diseases. Much has been written of this phase of the relationship, with evidence almost

wholly of statistical character. It would lead too far afield to enter into a lengthy discussion of the subject here. Lubarsch, summarizing the evidence in 1888, believed that while local tuberculous lesions, like other forms of chronic irritation, might contribute to the development of cancer, the relatively rare combination of tuberculosis and cancer could not be explained entirely by the difference in the usual age incidences of the two diseases, but that the feature of being comparatively rare in cancerous persons was one that tuberculosis shared with the acute infections. Boinet in 1907 could not find any decisive evidence pointing to an antagonism between cancer and tuberculosis; but Cleland in 1930 found in 1,305 autopsies an infrequent association of acute tuberculosis and cancer, which he explains on the basis of the rapid progress of the infection precluding the frequent appearance of cancer within the narrow time limits of the infection; however, in bodies that showed small healed tuberculous lesions cancer was distinctly less frequent than in bodies free from them. The validity of the relation would appear to be one that can be settled only by continued and comprehensive statistical study.

*Other Infections and Noninfectious Inflammatory Processes.*—As would be expected, other infections, especially those of chronic character, and other chronic noninfectious diseases have occasionally been observed as precursors of the local appearance of cancer. Von Hedry saw 2 cases of cancer of the mouth at sites of actinomycotic infection. Cases of sarcomatous change in chronic osteomyelitic lesions were reported by Beck, Harbitz and Simon. Even acute infections would appear at times to play a causative rôle; Luker cited a case of cervical cancer that appeared in a woman of 28 years, 5 months after a gonorrheal cervicitis, and Rohdenburg a case of perineal cancer after the same infection. Chronic inflammatory changes of noninfectious character are even more definitely established as precursors of cancer. Lynch saw skin cancer develop in connection with pellagrous dermatitis. Chronic mastitis, of undetermined origin, is notorious as an antecedent of cancer, a relationship that has been repeatedly observed, as by Fischer, among the earlier clinicians, in 1881, and especially in connection with acute carcinosis by Horn in 1911. That the mastitis may be in part of functional origin is suggested by the statistics of Leaf, who found that in no less than 71 of 73 cases of cancer of the breast there had been errors of lactation; and by the experiences of Lathrop and Loeb, who found an association between function and cancer in mice, with the more frequent occurrence of the latter in breeding animals. In the case of the stomach, the relationship of cancer to gastric ulcer is one to which attention was called long ago; a relatively early and comprehensive discussion is that of Hauser in 1883.



## COMMENT

It will be apparent that while a wide variety of irritants—chemical, physical and infectious—may be concerned in the causation of cancer, there is a marked diversity in the regularity with which their action is followed by neoplastic growth. In part this is unquestionably a matter of the agents themselves, in that they possess the power of cancerogenesis to varying degrees; in part it appears to be a matter of the individual, in that the likelihood of neoplastic response is an inconstant factor.

As to the mode of action of these irritants, there is little in the way of actual knowledge. The theories by which explanation has been attempted are numerous, and to the extent that they are matters of surmise, detailed discussion of them would be of questionable value. The theory of Ribbert, based largely on mechanical displacement, and that of Rowntree in explanation of roentgen cancer certainly do not meet all known facts. Similar hypotheses were advanced in regard to tar cancers by Itchikawa and Baum and by Ulesco-Stroganowa, while with these cancers a more active rôle was assigned to the epithelium by Murray, Sczcalik, Bang, Dreifuss and Bloch, Deelman, Champy and Vasiliu, and Borst, and Bierich took the view that the infiltrative growth is permitted by changes in the connective tissue induced by the liberation of lactic acid from the abnormal metabolism of the tumor cells. Abnormal relations of epithelial cells to their surroundings are also regarded as of fundamental importance in the theory of Burrows, who expressed the belief that cancerogenic agencies induce crowding of these cells, in which circumstance they accumulate archusia, a growth-stimulating principle, which he regards as more or less identical with vitamin B, while at the same time, in the case of tar cancer, through the agency of the tar there is a removal of growth-restraining material, which he termed ergusia and regarded as similar to if not identical with vitamin A. Turnbull viewed cancerous growth in conditions of irritation as being in the nature of response to strain, with increasing divergence from normal relations. Caspari and Crawford are inclined to see in the action of these irritants an effect wrought by necrohormones liberated by their activity. Rotter, dealing specifically with roentgen cancers, would interpret these as the result of stimulation of misplaced, extraregional sex cells by the irradiation, inducing in them partherogenetic development. A number of writers would regard the changes as being fundamentally alterations of surface tension, a phase of the theoretical explanations of cancer that will be discussed in some detail later. Bierich and Möller suggested that as cell protoplasm is by virtue of its colloidal dispersion an energetic system, with functional activity directly dependent on the degree of dispersion, study should be directed

to the energy-releasing or energy-furnishing power of the irritants, more especially of coal tar. Some experiments of Magrow are in line with this idea; he observed that under the influence of light of an extremely high frequency of vibration, cells begin to vibrate, and, with suitable vibration rates, enter into karyokinesis, which in conditions of abnormal equilibrium may increase to the point of induction of tumors. Hammett would see in all malignant tumors an increased metabolic activity as the result of accumulation of sulphydryl compounds, and in the case of tar cancers would ascribe the effect to the presence of suitable sulphur compounds in the tar, a theory that in a sense was anticipated by Green, who believed the distribution of cancer in the Orkneys was related to the use of sulphur-containing fuels. Finally, Carrel explains the induction of tumors in circumstances of chronic irritation as due to metabolic alteration, as a result of which the cells manufacture a specific substance that has the power of causing reproduction, and of imparting to the cells their malignant properties; some similar hypothesis would appear to be necessary to explain the property of transmission by cell-free filtrates shown by the fowl tumors that he produced by means essentially of chronic irritation.

An outstanding feature of the irritants in their induction of cancerous growth is their additive effect, to which allusion has several times been made in the preceding paragraphs. Berenblum, as a result of experiments on the causation of cancer by combinations of tarring and freezing with carbon dioxide snow, found that if the two agencies operate simultaneously and together, the effect is one of tumor inhibition, while if they act together but at different times, the usual additive effect is observed. Apparently the exact nature of the irritant is of little importance, provided it has a cancerogenic effect and acts at no time in too great intensity. It would seem that the effect of irritants in causing cancer is a gradual deviation from the normal, a deviation that occurs in response to forces at times of different character, and which, if Berenblum's results are correct, cannot be accomplished by too violent application of these forces.

Although it is not intended to discuss at this point the matter of predisposition to cancer, one phase of that subject needs brief consideration here. With practically all of the irritants mentioned in this section, age appears to play a rôle greatly subordinate to its usual importance in cancer incidence. Woglom would explain this by assuming that the effect of age on cancer incidence is merely that of allowing sufficient time for the development of cancer from some previous irritation. While it is possible that this explanation is correct, it is at best only a surmise, and until more definite knowledge is obtained of the factors that contribute to cancerous predisposition, it would be well to accept this as what it actually is—a hypothesis.

The relation of senescence to tumor development is not one that lends itself to direct experimental study, except in connection with the subject of the irritational cancers. That it is of an importance in lower animals fully equal to that in man has been shown by Goodpasture and Goodpasture and Wislocki, who were able to find frequent occurrence of multiple tumors in aged dogs. Goodpasture regarded them as being due to widespread degenerative changes of senile origin, from which certain cells escape by a process of dedifferentiation, with metaplasia and neoplastic growth of either benign or malignant character. In certain aspects—especially that of tissue imbalance from degeneration—this view is not entirely unlike that of Theilhaber, who regarded the tumors of senescence as due to disproportionate degeneration of connective tissue and epithelium, with even possible stimulation of the latter.

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#### CORRECTION

In the article by William Robinson, Ph.D., and Eloise Parsons, M.D., entitled, "Hemorrhage and 'Shock' in Traumatized Limbs: Changes in Total, Free and Bound Water of Blood and Muscle," in the December issue (**12**:869, 1931), the second line of the third and fourth columns of the table (page 883), bearing the caption "Free Water," should read 3.86 instead of 3.99 and 80.8 instead of 84.4, respectively.

## Notes and News

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**University News, Promotions, Resignations and Appointments, Deaths, etc.**—Eduard Kaufmann, emeritus professor of general pathology and pathologic anatomy in the University of Göttingen and author of the well known textbook on special pathologic anatomy, died on Dec. 15, 1931, in his seventy-first year.

Malcolm H. Soule has been promoted from associate to full professor of bacteriology in the school of medicine of the University of Michigan.

Vernon Kellogg has resigned as permanent secretary of the National Research Council, an office that he has held during the twelve years since the Council was placed on a peace-time basis.

Harold Main Vango, associate professor of medical jurisprudence in the University of Alberta, Canada, died on Dec. 29, 1931, from streptococcus infection in a puncture of the thumb received while making a postmortem examination.

David Bruce, a founder of tropical medicine, who discovered *Micrococcus melitensis* in 1887 and the transmission of *Trypanosoma gambiense* by the tsetse fly, has died at the age of 74. An Australian by birth, he was educated at the University of Edinburgh, joined the army medical service in 1883 and was assistant professor of pathology in the Army Medical School from 1889 to 1904.

Alfons Jakob, professor in the University of Hamburg and leader in the study of neuropathology, has died at the age of 47.

J. S. Young, lecturer in experimental pathology in the University of Leeds, has been appointed Musgrave professor of pathology in the University of Belfast.

Ward W. Summerville, Hannah research fellow in pathology in Western Reserve University, has been appointed associate professor of pathology in the University of Kansas.

Esmond R. Long, professor of pathology in the University of Chicago, has accepted the appointment as director of the laboratories of the Phipps Institute of the University of Pennsylvania.

**Society News.**The Fourteenth International Congress of Physiology will be held in Rome from Aug. 29 to Sept 2, 1932.

The Ninth International Congress of the History of Medicine will be held in Bucharest in September, 1932.

Edwin B. Fred has been elected president of the Society of American Bacteriologists; William Mansfield Clark, vice president, and J. M. Sherman, secretary-treasurer.

**Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.**—During 1931, the eighth year of the foundation, ninety-four applications for grants were received, thirty coming from this country and sixty-four from seventeen foreign countries. During the year twenty-six grants were made, nineteen for workers outside the United States. Eight of the 1931 grants were in aid of work on the general subject of nephritis. Applications for grants should be sent to Dr. Joseph C. Aub, 695 Huntington Avenue, Boston, before May 1, 1932.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

EXPERIMENTAL HEPATIC PIGMENTATION AND CIRRHOSIS. E. M. HALL and E. M. MACKAY, *Am. J. Path.* 7:327 and 343, 1931.

*Does Copper Poisoning Produce Pigmentation and Cirrhosis of the Liver?* In consideration of the well controlled experiments here presented, in which seventeen of twenty-one copper-fed animals showed pigmentation, many of them to a most extreme degree, and in view of the fact that nearly 50 per cent of this same group showed cirrhosis (this also being well marked and indisputable), we must conclude, in spite of the adverse reports by Flinn and Von Glahn, Polson, Herkel and others, that copper poisoning in rabbits produces pigmentation and cirrhosis of the liver in a high percentage of cases if adequate doses are given. This conclusion is in accord with the original work of Mallory and with the later investigation of Hall and Butt. We have found in most cases, at least under the conditions of our experiments, that from 75 to 100 mg. of copper must be stored for every 100 Gm. of wet liver tissue before pigmentation and cirrhosis result. The latter process apparently requires more time, and larger amounts of copper must be present in the liver tissue than are required to bring about pigmentation alone. That large quantities of copper are stored in the liver of the copper-fed animals as compared with that found in the controls is very evident on examination of table 1. None of our animals received carrots, mangelwurzels, turnips, cabbage or other plant substance rich in carotene; therefore the question of pigmentation due to these substances does not enter as a factor in the results presented here. The question of whether or not heavy feeding with carrots does produce pigmentation will be considered in a subsequent study. The resemblance of the lesions described in this paper to those of early hemochromatosis in man is very striking.

*The Effect of Heavy Carrot-Feeding on the Rabbit's Liver.*—Twenty-two rabbits were fed on diets containing a heavy percentage of carrots for from thirty-two to fifty days. Three animals developed Kupffer giant cells which contained a moderate amount of hemofuscin. One rabbit developed a definite early cirrhosis; two other animals showed slight connective tissue proliferation in their livers. Many of the animals showed clear, vacuolated cytoplasm in the liver cells probably due to excess glycogen storage. Other changes include parenchymatous degeneration, fatty infiltration and early nuclear degenerative changes. These conditions may be dependent on diet-deficiency due to the almost exclusive diet of carrots.

FROM AUTHORS' CONCLUSIONS AND SUMMARY.

EXPERIMENTAL COPPER POISONING. F. B. MALLORY and F. PARKER, JR., *Am. J. Path.* 7:351, 1931.

Acute poisoning with copper causes anemia, hemoglobinuria, necrosis of hepatic and renal cells and pigmentation. The pigment so formed is a combination of copper and some derivative of hemoglobin and can be stained with a neutral solution of hematoxylin. The differential staining property of this pigment depends on its copper content. The pigment granules, which often occur in the rabbit's liver under natural conditions and which certain other investigators have mistaken for those due to the action of copper, are not colored by this method. As a result of repeated injections of copper over a long period of time, a form of pigment cirrhosis results.

AUTHORS' SUMMARY.

## EXPERIMENTAL RENAL DISEASE PRODUCED BY X-RAY. M. S. S. EARLAM and A. BOLLIGER, J. Path. &amp; Bact. 34:603, 1931.

An experimental unilateral renal lesion has been consistently produced in the dog by a single direct exposure of the kidney to unfiltered x-rays of medium wavelength, equivalent to about 1,900r. The primary result of the irradiation is an acute degenerative lesion of the convoluted tubules, followed by scarring and regeneration, the relative proportions of which depend on the amount of initial damage. The degree of histologic change varies inversely with the weight of the animal, and in addition there is slight variation among individual dogs. Except in the smallest dogs, the irradiated kidney was capable by itself of supporting life through the period of maximum damage. The lesion is more gradual, less generalized and less standardized than that previously produced in this laboratory with double the dosage of x-rays. Provided that the opposite kidney is removed, various grades of renal disability can be produced, ranging from acute uremic death to practically complete recovery, the severity of the lesion varying inversely with the body weight of the dog. Irrespective of dosage, the irradiated kidney is able to support life by itself for a minimum period of thirty days. Nephritis produced by the x-rays, when well established, is characterized by nitrogenous retention, acidosis, hypercholesterolemia, polyuria, uremia and a variable amount of edema. The dosage of x-rays being in many instances sublethal, an appreciable degree of variation, independent of all other factors, was found to occur among individual dogs. The lesion, though not exactly duplicating any clinical syndrome, lends itself as a method for further research into many of the diagnostic, therapeutic or metabolic problems of nephritis.

AUTHORS' SUMMARY.

## CELLULAR CHANGES IN THE ALBINO RAT DUE TO MORPHINISM. WEN-CHAO MA, Chinese J. Physiol. 5:251, 1931.

This article gives a pictographic summary of the changes in the Golgi lipid and the mitochondria in acute and chronic morphinism of the white rat. The Golgi lipid may be regarded as a special material which is dissociated from the mitochondria and which is related to the fundamental mechanic activity of the cell. Stimulation with morphine results in the increase of the Golgi lipid and of the activity of the rat, but after a period of stimulation the activity falls and the amount of lipid in the cell is reduced. When lecithin is fed, neither the lipid nor the activity of the animal is reduced.

## THERAPEUTICS OF ECLAMPSIA BASED ON THE PATHOLOGIC PHYSIOLOGY. J. L. WODON, Arch. internat. de méd. expér. 6:245, 1931.

*Pathologic Physiology of Eclampsia.*—The characteristic disturbances of eclampsia are the paroxysmal convulsions, hypertension and the disturbance of the acid-base equilibrium of the blood. The end of a normal pregnancy is accompanied by a diminution of the alkali reserve which is accentuated during delivery. In spite of this difference of the alkali reserve the  $p_H$  of the blood remains within normal limits. In preeclampsia the alkali reserve is lower than at the end of a normal delivery, and there is an abnormal acid  $p_H$  indicating a disturbance of the acid-base equilibrium. At the time of paroxysmal convulsions and during coma, the disturbance of the acid-base equilibrium is extreme, the  $p_H$  averaging 7.07, the alkali reserve value being very low. In eclampsia an early hypertension is often observed, but the intensity is variable; it is essentially due to a spasm of the peripheral vascular system.

*Treatment for Eclamptic Convulsions with Magnesium and Calcium Salts.*—Calcium and magnesium when injected intravenously in therapeutic doses into decerebrated cats have similar action on the excitability of motor nerves. They elevate the rheobase and lower the chronaxia. The action of magnesium is the

more marked. Clinically, magnesium is given intramuscularly in doses of 100 mg. per kilogram of body weight, producing abolition of the eclamptic convulsions. Magnesium salt and calcium salt succeed in limiting the contraction in vitro of the uterus produced by different pharmacologic agents. In vivo, a therapeutic dose of calcium salts is incapable of abolishing uterine contractions, while magnesium salts show spasmolytic action comparable to opium. This pharmacologic property is confirmed by clinical observation. In therapeutic doses, calcium salts are incapable of weakening spasms of the peripheral vascular system produced experimentally, whereas magnesium salts have this property. In eclamptic patients, intramuscular injection of magnesium sulphate usually lowers the blood pressure 1 or 2 cm. of mercury.

*General Consideration.*—Treatment for eclampsia must aim at correcting two conditions: convulsions and acidosis. The acidosis of eclampsia formally contraindicates the use of chloroform, ether or chloral hydrate. Intramuscular administration of magnesium sulphate appears to be the treatment of choice. This "anticonvulsant drug" has a remarkable relaxing action on the spasms without lowering the alkali reserve and without lowering the  $p_H$  of the blood. It is advisable, on the other hand, to combat acidosis by bicarbonate washings.

#### AUTHOR'S SUMMARY.

EFFECT OF VIOSTEROL ON BONE. R. SOEUR, Arch. internat. de méd. expér. 6:365, 1931.

Administration of massive doses of viosterol is toxic; it embarrasses the general state of the animal and can produce rapid death. As shown by other investigators, these conditions are closely allied with calcium metabolism, producing intensive mobilization of calcium with a negative balance in the bones. The blood calcium tends to become elevated, and there results in various organs (heart, arteries, veins, etc.) abnormal storage depots of calcium. This mobilization of calcium is produced at the expense of the bone. It is the latter that is the subject of the present study. Using young and adult guinea-pigs and also adult rats, we have confirmed the observations of previous workers concerning toxicity, the blood calcium and phosphorus values and abnormal calcification. We have studied the bones from a standpoint of pathologic anatomy. In the ribs, vertebra, skull and long bones we have observed the following: the reabsorption of bone with resulting fractures, abnormal phenomena in bone marrow, manifested by intensive vascularization and aseptic necrosis and arrest in the osteogenic activity in young animals. We have obtained microscopic pictures absolutely different from those produced by the administration of parathyroid extract (generalized bony fibrosis). It is logical for us to suppose that viosterol does not act by exciting the parathyroid gland, and that the action in large doses is simply a toxic effect.

#### AUTHOR'S SUMMARY.

EXPERIMENTAL ACUTE HEMATOGENOUS NEPHRITIS. W. CHRIST, Beitr. z. path. Anat. u. z. allg. Path. 85:221, 1931.

Hämäläinen found that the intravenous injection of staphylococci into rabbits is followed by the formation of bacterial colonies within the intertubular capillaries. This finding was in accord with that of Albrecht and other previous workers, but was contrary to the still older belief of Orth that the acute nephritis due to the presence of bacteria in the blood stream is an excretion nephritis set up by the passage of the bacteria through the glomerular vessels. In Hämäläinen's experiments, no intracapillary localization of bacteria could be detected by histologic methods in animals killed at intervals up to the eighteenth hour after injection, although the urine had yielded positive cultures of the injected staphylococci from the ninth hour on. Christ repeated the short duration experiments, intro-

ducing a slight modification of technic, the aim of which was the detection of bacterial localization at short intervals following the intravenous injection of the bacteria. When the animals were killed at the desired periods after injection, the kidneys were removed under aseptic precautions. One half of each kidney was immediately fixed for histologic study. The other half was incubated in a moist chamber, portions being removed and fixed at various periods up to eighteen hours. Using this procedure, Christ was able to detect the intracapillary localization of the bacteria as early as six hours following injection. The localization occurred in the intertubular capillaries of the corticomedullary junction, of the medulla and of the papilla. Passage of bacteria into the tubules and involvement of the glomeruli were secondary to the intertubular intracapillary localization. Primary localization of the cocci within the glomeruli, which leads to the formation of abscesses in the cortex, could be brought about experimentally only when the suspension of bacteria used for injection contained clumps too large to pass through the glomerular tuft vessels.

O. T. SCHULTZ.

*HYPERGLYCEMIA IN EXPERIMENTAL INFECTION.* T. FUKUDA and KO ITABASHI, Ztschr. f. d. ges. exper. Med. **76**:756, 1931.

Hyperpyrexia and hyperglycemia are not always concomitant; they are two entirely independent phases of infection. Hyperglycemia is an indication of moderate severity of infection. In slight and in overwhelming infections a rise in the blood sugar does not take place. After sectioning of the splanchnic nerves a rise in blood sugar does not occur. The authors conclude from their experiments that the hyperglycemia of infection is a central effect on the sugar center of the brain and not a peripheral action on the suprarenals or liver.

SANDER COHEN.

*ANIMAL EXPERIMENTS ON THE LOCALIZATION OF BLOOD RESERVOIRS.* A. KAHLSTORF and H. LUDWIG, Ztschr. f. d. ges. exper. Med. **76**:804, 1931.

India ink injected intravenously into rabbits and guinea-pigs collects slowly in the spleen. The blood found there does not take much part in the circulation. In shock produced by peptone and in chloroform anesthesia, conditions that decrease the volume of the circulating blood, the india ink is found in the spleen in large quantities. The conditions leading to a filling of the splenic reservoir exert a similar effect on the liver capillaries, which can also be designated as a blood reservoir. Of the conditions that increase the circulating blood mass, rise in external temperature is without effect on the filling of the spleen and liver with india ink.

SANDER COHEN.

### Pathologic Anatomy

*MECKEL'S DIVERTICULUM.* AMOS CHRISTIE, Am. J. Dis. Child. **42**:544, 1931.

A short history of the literature on Meckel's diverticulum is presented, and a series of 63 such cases is reported. The embryology is discussed as a basis for a better understanding of the anatomy and pathology of the diverticula of the terminal ileum. Comparisons with previous groups substantiate observation of a preponderance of males (74.6 per cent), and set the probable incidence of Meckel's diverticulum at 1 per cent in a large series (5,768) of necropsies. The site of Meckel's diverticulum in relation to the ileocecal valve varies within wide limits. Types of Meckel's diverticulum are discussed and illustrated. The complications are listed, and the coincidence of other anomalies associated with Meckel's diverticuli in 33 per cent of the cases is offered as evidence of a defective germ plasm as an etiologic factor.

AUTHOR'S SUMMARY.



PERSISTENT DUCTUS OMPHALOMESENTERICUS. M. L. BRIDGEMAN AND F. R. MENNE, *Am. J. Dis. Child.* 42:602, 1931.

A case of persistent ductus omphalomesentericus in a boy, aged 8, is presented. An acute Meckel's diverticulitis with stenosis of the lower end of the ileum followed by a spontaneous ileosigmoidostomy is delineated. Such short-circuiting of the contents of the bowels leads to an unusual altered physiologic condition that may result fatally, due to peritonitis or obstruction.

AUTHORS' SUMMARY.

TOPOGRAPHIC ANATOMY AND HISTOLOGY OF THE VALVES IN THE HUMAN HEART. L. GROSS AND M. A. KUGEL, *Am. J. Path.* 7:445, 1931.

There has been described in this paper in some detail the normal histology and topography of the individual cusps of the valves in the human heart. It has been shown that these have a number of characteristics in common and yet sufficient individual differences to distinguish them from one another. The desirability of sharply delimiting the different cusps has been discussed and a method of accomplishing this has been suggested. This method is calculated to clear up the confusion that exists on the question of the presence of myocardium in the auriculoventricular valves. A simple but comprehensive classification of the various valve layers has been given. In designing this classification, there has been borne in mind the advisability of using a uniform nomenclature and of reducing the variations in valve structure to as few common denominators as possible. Attention has been drawn to the possible bearing that the various differences in valve structures may have on the development of pathologic processes which occur at these sites. An indication has also been given of the evolutionary and involutionary postnatal changes that take place in the cusps. The main purpose of the work here reported is to serve as a base line for further studies on disease of heart valves, because it is realized that an intimate knowledge of the structure of the valve cusps, together with their normal variations and age-period changes, is essential for the recognition and understanding of pathologic processes.

AUTHORS' SUMMARY

INFLAMMATORY ADENOMATOID HYPERPLASIA OF THE MAJOR DUODENAL PAPILLA IN MAN. V. J. DARDINSKI, *Am. J. Path.* 7:519, 1931.

In 54 per cent of 100 cases in which autopsy was performed, definite changes were found in the major duodenal papilla. The changes apparently were benign and did not cause definite symptoms. Enlargement of the ends of the folds and thickening of the papillary wall were due to the presence of hyperplastic mucous glands, resulting probably from repeated attacks of inflammation at the outlet of the papilla.

AUTHOR'S SUMMARY.

THE ELASTIC LAYER IN THE CEREBRAL VESSELS OF THE NEW-BORN AND OF CHILDREN. C. R. TUTHIL, *Arch. Neurol. & Psychiat.* 26:268, 1931.

The cerebral vessels were examined in two stillborn fetuses and in twenty-four children, varying in age from birth to 14 years, for the condition of the elastic membrane. In some children the elastic layer fails to develop normally, and this Tuthil thinks may account for the frequency of cerebral hemorrhages in the new-born. The thickness of the elastic layer at birth and the period of its active growth depends on the size of the vessel. It shows in some cases splitting at and before the branching of the small or large vessels; the splitting is associated in some cases with formation of knotty areas.

GEORGE B. HASSIN.

INTRACRANIAL CALCIFICATION. C. B. COURVILLE AND L. J. ADELSTEIN, Arch. Surg. **21**:801, 1930.

Calcareous deposits are common in intracranial neoplasms, especially in the gliomas. The calcification is probably due to necrosis with secondary deposits of calcium in the blood stream. Calcification of the small blood vessels of tumors as well as generalized calcification of the media and intima of intracranial vessels is common. Frequently cysts result from calcific occlusion of small blood vessels. This calcification may be detected roentgenologically, and is of some diagnostic importance. Calcification in tumors is generally quite uniform. Calcification in neoplasms bears no important relationship to the degree of malignancy of the tumor.

N. ENZER.

ILEOCECAL CYSTS. E. DRENNEN, Arch. Surg. **22**:106, 1931.

Three instances of ileocecal cysts, two in children 9 months and 2 years of age, respectively, and one in a woman 21 years of age, are recorded. The cysts were lined by columnar epithelium and were about 3.5 by 1.5 cm. in size. Twenty-three cases were found in the literature. Such cysts seem to be of developmental origin.

N. ENZER.

CONGENITAL ABSENCE OF THE PERICARDIUM. C. S. BECK, Arch. Surg. **22**:282, 1931.

Complete absence of the pericardium was found at autopsy in a colored woman 56 years of age. The left ventricle was hypertrophied. There was a moderate degree of arteriosclerosis, and the patient had had hypertension. The right auricle was adherent to several rudimentary bands or folds of epicardium. There seemed to be no impairment of the freedom of heart action.

The question arises as to what is the function of the pericardium. Adhesions between the heart and adjacent structures occur more frequently in the absence of the pericardium. There are no cases on record in which the absence of the pericardium seemed to account for death. There is no evidence that it is of any benefit in a dilated heart.

N. ENZER.

INTERVERTEBRAL DISK EXTENSIONS INTO THE VERTEBRAL BODIES AND THE SPINAL CANAL. D. SASHIN, Arch. Surg. **22**:527, 1931.

Trauma and chronic arthritis are the commonest causes of degeneration of the intervertebral cartilaginous disks. These may extend into the vertebral bodies and even into the spinal canal and cause compression of the spinal cord.

N. ENZER.

ATROPHIC FIBROSIS ASSOCIATED WITH LYMPHOID TISSUE IN THE THYROID. A. GRAHAM AND E. P. McCULLAGH, Arch. Surg. **22**:548, 1931.

Thyroids in this group are firm, usually uniform and not nodular, frequently firmly attached to the surrounding structures. They are most common in women after the menopause, and without any symptoms significant of change in thyroid function. The regional lymph nodes are not involved, and there is no evidence of metastasis. The general health is good. There is no evidence of any local acute or subacute inflammatory reaction. The striking anatomic feature of the gland is the generalized, diffuse lymphocytic infiltration, with some follicular formation and with moderate compression atrophy of the thyroid acini. These acini, when crowded together, sometimes give the appearance of slightly hyperplastic epithelium.

N. ENZER.

PRIMARY ISOLATED LYMPHOGRANULOMATOSIS OF THE STOMACH. H. A. SINGER, Arch. Surg. 22:1001, 1931.

Six cases of primary Hodgkin's disease of the stomach have been recorded in the literature. Five of the patients recovered after operation and remained well. Six died a postoperative death. Postmortem examination failed to reveal any other granulomatous lesions elsewhere in the body. The author reports a seventh case which likewise failed to reveal any lesion elsewhere in the body.

N. ENZER.

STILL'S DISEASE. R. REITANO, Arch. ital. di anat. e istol. pat. 1:489, 1930.

The literature on Still's disease is summed up. A case which had lasted three years is described in a woman of 18. Clinically, there was stiffness of the wrist and sternoclavicular joints. The latter were deformed. The spleen, increased in volume, presented atrophy of the follicles, congestion and hyperplasia of the reticulum cells. The various lymph gland systems were diffusely swollen; histologically they presented partly typical tuberculous lesions, partly slight reduction of the follicles, increase of the reticulum cells, desquamation of the sinuses and hyalinosis of the stroma. In some joints, with macroscopically visible erosions, there were regressive changes in the cartilage, and even necrosis and substitution with fibrous tissues. Tuberculous areas were found in the lungs and liver. The author considers Still's disease as a morbid syndrome of multiple etiology; in the case described there probably existed a tuberculous factor.

G. PATRASSI.

THE REGRESSION OF DUCTUS BOTALLI IN MAN. A. COSTA, Cuore e circolaz. 14:546, 1930.

The minute histologic structure of the duct and its involution have been investigated in sixty cases, including embryos in various stages and new-born infants. During fetal life the intima is thick; has little elastic tissue, the muscular cells being arranged lengthwise and rich in mucoid matter. The muscular wall proper has a circular inner layer and a longitudinal outer layer of muscle cells and contains elastic tissue and some mucoid matter. This type of structure should favor the discharge of the blood from the pulmonary artery to the aorta. After birth the lumen of the duct grows narrower because of an elastic hyperplasia of the muscular wall and of the internal elastic coat and the intima. The mucoid material diminishes. The anatomic closing is completed by the formation in the lumen of blood clot with calcium and iron. This precipitation of minerals is followed by a fibrous elastic sclerosis of the walls of the duct with atrophy of the muscle cells. Chronologically, the processes differ in different persons.

G. PATRASSI.

THE GENESIS OF EOSINOPHILIA. F. PESCATORI, Riv. di pat. e clin. d. tuberc. 4:735, 1930.

Experimenting on white mice, the author observed that a pneumothorax regularly causes eosinophilia, and that the latter reappears regularly every time that gas is introduced. The histologic examination of the collapsed lung showed that there was pulmonary and peribronchial eosinophilia, extensive accumulations of blood pigment and an intense proliferation of the bronchial and peribronchial histiocytes. Such results correspond to those observed by the author in the lungs of persons who had died during an attack of bronchial asthma, and with those experimentally obtained in the lungs of rabbits in which bronchial spasm had been caused by extraneous bodies. According to the author, these observations confirm the hypothesis that both bronchial and hematic eosinophilia is to be ascribed to

asphyxial phenomena, local and general; and that it is not a morphologic expression of immune reactions as some one has asserted.

G. PATRASSI.

MICROGLIA. G. DADDI, *Sperimentale*, Arch. di biol. 85:5, 1931.

By experiments the author has reexamined the problem of the ectodermic or mesodermic origin of the microglia by searching whether it has fat-storing properties or not. After injecting small quantities of olive oil into the carotid artery of rabbits, he verified that, in about three hours, numerous small drops of fat had appeared in the body of microglia cells near to embolized capillaries. Such cells were slightly hypertrophic. In this stage there were no destructive phenomena in the nervous matter, which might explain the presence of fat in the microglia. After fourteen hours, granules of fat appeared in the bodies and processes of astrocytes in contact with the walls of certain vessels; also the endothelial and the adventitial layers of the latter were stained by the specific coloring matter for fats. After eighteen to twenty-four hours there was no trace of fat in the microglia nor in the astrocytes; there were localized destructive phenomena caused by emboli. It is concluded that, most probably, the microglia cells (and perhaps also the histiocytic glia) possess fat-storing properties similar to those of the reticulohistiocytes.

G. PATRASSI.

THE BLOOD IN LYMPHOGRANULOMATOSIS. GÜNTHER STRAUBE, *Folia haemat.* 44:125, 1931.

There is no single or significant diagnostic blood finding in lymphogranulomatosis, and it is not possible through hematologic investigation alone to arrive at the diagnosis.

But the blood picture in addition to other occurrences in lymphogranuloma might be of diagnostic importance, entirely aside from the fact that it sharply separates the leukemias from Hodgkin's disease.

FROM AUTHOR'S SUMMARY.

BILATERAL APLASIA OF THE KIDNEYS. R. ROSENBAUM, *Frankfurt. Ztschr. f. Path.* 41:136, 1931.

Ninety-one such cases are collected from the literature, described in short, and one new case is added. This was the case of an 11 day old male premature infant. Both kidneys and both ureters were absent; the urinary bladder was found in a normal position. It was a small organ with very thin walls. No other abnormalities could be discovered, and no changes were found in the lower extremities. The possibilities leading to aplasia of the kidneys are discussed.

OTTO SAPHIR.

SPECKLED SPLEEN (MULTIPLE NECROSIS IN THE SPLEEN) AND ITS RELATION TO ARTERIOLAR SCLEROSIS. B. SPIER, *Frankfurt. Ztschr. f. Path.* 41:160, 1931.

Cases of a moderate degree of arteriolar sclerosis, as a rule, show no changes in the spleen. Cases that show marked sclerosis, however, reveal nutritional disturbances of the parenchyma that interfere with the normal function of the hematopoietic apparatus. The changes in the spleen are not caused by formation of secondary thrombi within the arterioles but are produced directly by changes within the wall of the arteries (arteriolar necrosis). Such a case is reported in a 51 year old woman who died of encephalomalacia. A series of cases of the malignant type of arteriolar sclerosis with renal and pancreatic changes are also reported and the changes in these organs correlated with those found in the spleen of the first case.

OTTO SAPHIR.

CYLINDRIC NARROWING OF THE DESCENDING PART OF THE THORACIC AORTA AS A RESULT OF A MESAORTITIS. W. HICKL, Frankfurt. *Ztschr. f. Path.* **41**: 176, 1931.

Such a case is reported in a 38 year old woman. The stenotic portions in the aorta measured 6 cm. in length and 2.4 cm. in width. The cause of the stenosis is a chronic fibrous mesaortitis with a marked new formation of connective tissue in the adventitia. It is possible that syphilis was the underlying cause of the mesaortitis; it is more likely, however, as the author states, that the stenosis was the result of a nonspecific mesaortitis which might have had the same etiology as articular rheumatism, from which the patient had suffered two years previously. This explanation seems the more plausible because of the simultaneous finding of a moderate stenosis of the mitral orifice. The author believes that this case is unique in the literature.

OTTO SAPHIR.

SYSTEM HYPERPLASIA OF THE RETICULO-ENDOTHELIAL SYSTEM. S. M. DERISCHIANOFF, Frankfurt. *Ztschr. f. Path.* **41**:184, 1931.

The author proposes the following classification of the pathologic hyperplasia of the hematopoietic apparatus.

I. Hyperplasia of the parenchyma

1. Leukoses

- (a) Lymphatic and myeloid leukoses
- (b) Lymphatic and myeloid aleukoses
- (c) Monocytic leukemia (Schilling)

2. Erythremia

3. Parenchymatous tumors

- (a) Myeloma
- (b) Chloroma
- (c) Lymphosarcoma
- (d) Plasmocytoma

II. Hyperplasia of the stroma (reticulo-endotheliosis)

1. In infectious diseases

- (a) Desquamative catarrh of the lymph node
- (b) Large cell hyperplasia in tuberculosis
- (c) Large cell hyperplasia in typhoid fever
- (d) Syphilis
- (e) Sporotrichosis
- (f) Mycosis fungoides and other diseases

2. Lymphogranulomatosis (typical and atypical forms)

3. Reticulo-endotheliosis in cases of disturbances of the metabolism

- (a) Gaucher's disease
- (b) Niemann-Pick's disease
- (c) Xanthomatous metamorphosis of the reticulo-endothelium in diabetes

4. Blastomatosis with reticulo-endotheliosis

5. Reticulo-endotheliosis per se

The author reports a case which, according to his opinion, is a case of reticulo-endotheliosis per se with symptoms of pernicious anemia. This is a case of a 26 year old woman. The blood picture was as follows: red blood corpuscles, 650,000; leukocytes, 7,000; hemoglobin, 30 per cent; color index, 1.5. There was a moderate poikilocytosis. Erythroblasts were present, some of which showed outspoken basophilic granules. A splenectomy was performed, but the patient died two hours afterward. The spleen weighed 2,400 Gm., and measured 12 by 17 by 32 cm. It was dark gray and revealed several infarcts. There also was an accessory spleen measuring 4 by 4 by 4 cm. Histologically, many reticulum and

endothelial cells were found in all the blood-forming organs. They disclosed iron-containing pigment, remnants of erythrocytes and leukocytes. The lymph nodes showed large cells which resembled Gaucher cells. The bone marrow was not examined.

OTTO SAPHIR.

### Pathologic Chemistry and Physics

THE MICROCHEMICAL DEMONSTRATION OF COPPER IN PIGMENT CIRRHOSIS. F. B. MALLORY and F. PARKER, JR., *Am. J. Path.* 7:365, 1931.

The presence of copper was demonstrated in islands of regeneration in five active cases of pigment cirrhosis by means of the hematoxylin test. Its presence in the sections was confirmed by the triple nitrite test in two of the four cases tested. The copper occurred in pigment granules in the young liver cells and in masses of inspissated bile. After causing the deposition of a copper hemoglobin compound, the copper is quickly eliminated in the bile and therefore does not accumulate in the liver. The pigments (copper-hemofuscin, hemofuscin and hemosiderin), derived successively from hemoglobin and containing masked and unmasked iron, require years to transform them. As a result, they accumulate in large amounts in the liver and form the most prominent feature of this type of cirrhosis. The necrosis of liver cells, which eventually results in cirrhosis, is apparently due to the toxic action of copper and not to the mechanical presence of the pigments. Proof has been presented that copper is present in the early hemofuscin pigment granules, in the excretion of bile and also in excess of normal in the liver tissue as evidence that chronic poisoning with copper causes hemochromatosis.

AUTHORS' SUMMARY .

A COLORIMETRIC MICROMETHOD FOR THE DETERMINATION OF SULPHUR IN DEPROTEINIZED BLOOD FILTRATE. I. S. LORANT and L. KOPETZ, *Biochem. Ztschr.* 238:67, 1931.

The sulphur content of 5 cc. of blood is determined first. After deproteinization and ashing, the sulphur is first transformed into sulphate, which is reduced to hydrogen sulphide. After the distillation of hydrogen sulphide, the sulphur is determined with the highly specific Caro's reaction as methylene blue (methylthionine chloride, U. S. P.). The total sulphur of the normal human blood was found with the method to be in the mean 6.8 mg. per hundred cubic centimeters.

WILHELM C. HUEPER.

A SIMPLE TITRIMETRIC METHOD FOR THE DETERMINATION OF THE ALKALI RESERVE IN SERUM. F. ELLINGER, *Biochem. Ztschr.* 238:80, 1931.

One cubic centimeter of human serum diluted with 4 cc. of normal sodium chloride solution is titrated with tenth normal hydrochloric acid, bromthymolblue being used as an indicator. Hydrochloric acid is added drop by drop under shaking till a  $p_H$  of 6 is reached. The Walepole comparator and Soerensen's buffers are used. The sodium salts of the intermediary metabolic products do not appear in this titration of the alkali reserve, which is represented by the bicarbonate ( $HCO_3^-$ ) content and the acid-combining power of the serum. The alkali reserve is expressed in cubic centimeters of normal acid.

WILHELM C. HUEPER.

HEMAGGLUTINATING AND HEMOLYTIC QUALITIES OF BILE PIGMENTS. A. CLEMENTI and F. CONDORELLI, *Biochem. Ztschr.* 238:278, 1931.

Bilirubin possesses marked hemagglutinating and hemolytic qualities on erythrocytes suspended in physiologic solution of sodium chloride. The hemagglutinating action precedes the hemolytic action of the bilirubin. The hemagglutinating

action may still be present at concentration in which the hemolytic action has already disappeared. The lowest concentration in which the hemagglutinating action of bilirubin is still demonstrable lies around 1:20,000. The hemagglutinating and hemolytic qualities of biliverdin are by far less than those of bilirubin.

WILHELM C. HUEPER.

THE SUBFRACTIONS OF GLOBULIN AND ALBUMIN IN TRANSUDATES OF LIVER CIRRHOSIS, SARCOMA and CARCINOMA. B. LUSTIG, *Biochem. Ztschr.* **238**: 307, 1931.

The total amount of albumin in carcinomatous transudates is considerably higher than that found in transudates due to cirrhosis or sarcoma. The amount of globulin soluble in NaOH is from three to four times higher than in cirrhosis, while the amount of the globulin soluble in sodium chloride is only about one sixth of that found in cirrhotic fluid. The carbohydrate content of the globulins soluble in NaOH of cancerous fluids increased in comparison to the other globulins. The free COOH- and NH<sub>2</sub>- groups are lowered in the cancerous globulins soluble in sodium chloride. The globulins soluble in NaOH have a markedly increased content of carbohydrates, considerably lowered content of nitrogen and an almost equal content of NH<sub>2</sub>- and COOH- groups. The increase of the globulins soluble in NaOH and their increased content of carbohydrates in carcinoma explains the increased sugar values found in cancerous serum after hydrolysis.

WILHELM C. HUEPER.

DETERMINATION OF UREA IN URINE WITH A SUBLIMATE METHOD. B. LUSTIG and H. BROMBERG, *Biochem. Ztschr.* **238**:321, 1931.

A clinically useful method of the determination of urea in urine is described, in which, following a tungstic acid precipitation, urea is determined with the help of a sublimate titration. The method is accurate to  $\pm 4$  per cent and requires, as asserted, only a little time for its performance.

WILHELM C. HUEPER.

THE METHODS OF URIC ACID DETERMINATION IN BLOOD SERUM. A. BLANKENSTEIN, *Biochem. Ztschr.* **238**:461, 1931.

In parallel determinations of uric acid in blood serum after Thannhauser, Folin, Folin and Svedberg, Benedict, Morris, Vladescu and Flatow the relatively best agreeing results were obtained by the methods of Thannhauser, Folin and Flatow. On addition of uric acid and after deproteinization, 80.6 per cent of the added uric acid was recovered if the deproteinization method of Folin was used, 68 per cent, if Thannhauser's method was used and only 53 per cent if the proteins were precipitated by uranyl acetate. If the serum was deproteinized after Folin and the colorimetric method of Thannhauser was used, 101 per cent of the added uric acid was recovered. It is therefore recommended to use for the determination of uric acid in serum the deproteinization method of Folin combined with the colorimetric method of Thannhauser.

WILHELM C. HUEPER.

### Microbiology and Parasitology

UPPER RESPIRATORY DISEASE (COMMON COLD) AND THE WEATHER. WILLIAM M. GAFAFER, *Am. J. Hyg.* **13**:771, 1931.

The evidence presented strongly suggests that changes in weather during a warm season are associated with the incidence of disease in the upper respiratory tract (common cold) more than are changes in weather during a cold season.

AUTHOR'S SUMMARY.

THE SUSCEPTIBILITY OF CHICK EMBRYOS TO THE FOWL-POX VIRUS. A. M. WOODRUFF and E. W. GOODPASTURE, *Am. J. Path.* **7**:209, 1931.

Ectodermal and entodermal cells of the chorio-allantoic membrane of the chick, as well as embryonic chick skin, are susceptible to infection with the virus of fowl-pox at an early stage in the development of the embryo. Whether or not this specific susceptibility is acquired as a result of cellular differentiation has not been determined. Four methods for the isolation of uncontaminated fowl-pox virus are described. In two of these methods the virus is developed in tissue that has never been contaminated by extraneous micro-organisms. Fowl-pox infection has been induced in the trachea of the adult hen by inoculation with uncontaminated virus.

AUTHORS' SUMMARY.

THE TRANSMISSION OF DENGUE IN SUMATRA. E. P. SNIJDERS, E. J. DINGER and W. A. P. SCHÜFFNER, *Am. J. Trop. Med.* **11**:171, 1931.

It is possible to dispatch mosquitoes of the genus *Aedes*, infected with the virus of dengue, on long distances (Sumatra to Amsterdam) without destroying their infectivity. It has been proved possible to cause typical cases of dengue in Holland through bites from mosquitoes infected in the Dutch East Indies (Sumatra). The endemic dengue found in Sumatra is transmitted by *Aedes aegypti*, as well as by *Aedes albopictus*. Accordingly, for the endemic Sumatran dengue, we have been able to confirm the findings of Cleland for Australia, Siler for the Philippine Islands and Blanc for Greece, viz., that the dengue fevers are transmitted by *Aedes aegypti*. Moreover, we were able to confirm the finding of Simmons for the Philippine Islands, viz., that *Aedes albopictus* is also a transmitter. We were able to increase the conclusive force by carrying out the experiments in a place where spontaneous infection is absolutely excluded. The symptomatology of dengue is more variable than is often conceived.

AUTHORS' SUMMARY.

CYSTINURIA AND TUBERCULOSIS. HOWARD B. LEWIS and MARIE F. O'CONNOR, *Am. Rev. Tuberc.* **23**:134, 1931.

Careful examination of the urine of some 150 tuberculous patients failed to demonstrate any relationship between tuberculosis and cystinuria as maintained by Monceaux. The tests used (Sullivan, Brand, Looney, Gaskell) were shown by control experiments to be sufficiently delicate to indicate the presence of cystine in small amounts.

H. J. CORPER.

THE EFFECT OF IRRADIATED ERGOSTEROL ON CALCIFICATION OF TUBERCLES. TOM DOUGLAS SPIES, *Am. Rev. Tuberc.* **23**:169, 1931.

Repeated large doses of activated ergosterol administered to rabbits with chronic tuberculosis produce, as in acute tuberculosis, calcification within the necrotic and caseous portions of the tubercles.

H. J. CORPER.

BLOOD GROUPS IN TUBERCULOSIS. K. T. SASANO, *Am. Rev. Tuberc.* **23**:207, 1931.

The percentages of the blood groups in 1,000 cases of tuberculosis agree closely with those of Karsner and Koeckert. In the past three years, 3,082 tests on 1,000 persons revealed no evidence of a change in the blood group of any person. There was not found any specific relation of the blood groups to any particular disease. There was no evidence of a greater resistance or susceptibility to tuberculosis with any one of the blood groups. The response to treatment appeared to be better among the tuberculous persons in group A than among those in group O.

H. J. CORPER.



THE LYMPHOCYTIC-MONOCYTIC RATIO IN EXPERIMENTAL TUBERCULOSIS IN GUINEA PIGS. EDWARD B. BOSWORTH, *Am. Rev. Tuberc.* **23**:318, 1931.

In guinea-pigs inoculated with a virulent strain of tubercle bacilli a blood picture developed characterized by a high percentage of monocytes and a low percentage of lymphocytes. The ratio between these percentages and the lymphocytic-monocytic index is correspondingly low. Guinea-pigs inoculated with an avirulent strain of tubercle bacilli overcome the infection and present a blood picture but slightly different from that of uninfected animals and in striking contrast to that presented by animals dying of a virulent infection. The lymphocytic-monocytic index in experimental tuberculosis in guinea-pigs is of value in determining the reaction of the animal to its infection.

H. J. CORPER.

TYPHUS FEVER. H. PINKERTON, *J. Exper. Med.* **54**:181 and 187, 1931.

Study of two strains of epidemic (European) typhus and two strains of endemic (American) typhus in laboratory animals has shown their essential identity. The characteristic lesions of the brain can be shown to occur with all four strains after subcutaneous inoculation, and Rickettsiae-filled cells can be found in the serotal sac with all four strains after intraperitoneal inoculations. A strain of European typhus that for eight years had yielded no obvious serotal reaction in guinea-pigs afterward gave rise to a periodic serotal reaction of variable severity. At present it occupies an intermediate position in this respect between the recently isolated European strain and the American strain. Variations in the serotal reaction in guinea-pigs appear to be correlated with variations in the virulence of strains in the human host. The period of incubation in guinea-pigs after intraperitoneal inoculation depends largely on the number of Rickettsiae injected.

A satisfactory method is described for the topographic study of *Rickettsia prowazeki* in sections of the serotal sac of typhus-infected guinea-pigs. In such sections, Rickettsiae are always intracellular. The Rickettsiae of typhus multiply luxuriantly in the serosal cells and produce great distention of these cells. Rickettsiae may be found in small numbers in the endothelial cells lining the underlying capillaries in the testes and scrotum, but are not seen in perivascular macrophages, connective tissue cells, fat cells, smooth or striated muscle fibers or epithelial cells. Rickettsiae are rarely phagocytosed in small numbers by polymorphonuclears, but are never seen in lymphocytes, plasma cells or eosinophils. When infected mesothelial cells proliferate and desquamate, they rapidly lose their content of Rickettsiae. The large mononuclear cells seen in smears of the exudate of the serotal sac may be separated into two groups: (1) the serosal (mesothelial) cells, which become heavily infected with Rickettsiae, but which are not phagocytic for graphite ink, and (2) the macrophages (phagocytic cells), which do not contain Rickettsiae.

AUTHOR'S SUMMARIES.

BLOOD CHEMICAL CHANGES IN STREPTOCOCCUS SEPTICEMIA. R. W. LINTON, *J. Exper. Med.* **54**:223, 1931.

The following changes have been found to occur in rabbits given fatal intravenous doses of *Streptococcus hemolyticus*: The blood sugar concentration drops at a constant rate throughout the disease, but does not reach a condition of hypoglycemia. Glycogen is present in the liver at death. The carbon dioxide-combining capacity is lowered markedly at first, then returns to a somewhat higher level, at which it continues until the terminal stage of the disease, when the acidosis becomes very marked. The concentration of inorganic phosphorus is markedly increased at the terminus of the disease. This increase is greater in animals showing an acute course than in those in which the disease is of the fulminating type. Calcium also shows terminal changes, decreases occurring in both groups. In the acutely infected rabbits the decrease is less than in the fulminating group, although in both a pathologic level is reached. Nonprotein nitrogen and creatinine are greatly

increased in the terminal stages, in both groups of animals. It is suggested that these observations can be explained on the assumption that a large amount of acid is produced by the streptococcus in vivo.

AUTHOR'S SUMMARY.

EXPERIMENTAL "MAD ITCH." R. E. SHOPE, J. Exper. Med. **54**:233, 1931.

The clinical picture and the gross pathologic changes of spontaneous and experimental "mad itch" are described, and the inciting agent is shown to be a filtrable virus. It has been possible to prepare virucidal serum capable of neutralizing the virus. Fatal infections are regularly produced in rabbits when the virus is administered subcutaneously, intracerebrally, intravenously, intratesticularly, intraperitoneally or intranasally, or when it is dropped on a scarified area of skin. Its infectivity for other species by various routes is reported. The rabbit, guinea-pig, white rat, white mouse, gray field mouse, cow, cat, duck, chicken and hog are susceptible to experimental infection. The disease is not contagious under laboratory conditions, and in the animal body the virus is restricted largely to the region of inoculation and the lung. The virus can be stored for relatively long periods in 50 per cent glycerol or in the dried state. A comparison of "mad itch" with pseudorabies leads to the tentative conclusion that the inciting agents of both are the same, although the strains of the two viruses that are under study possess readily demonstrable differences.

AUTHOR'S SUMMARY.

RHINOSPORIDIUM KINEALYI [SEEBERI] INFECTION. P. K. KURUP, Indian M. Gaz. **66**:239, 1931.

Two cases are reported, one occurring in the pharynx of a woman, aged 24, and the other in the upper eyelid of a boy, aged 12. In both instances the peduncular growths were removed by excision.

GUINEA-PIG EPIDEMIC DUE TO STREPTOCOCCUS. W. W. BEATTIE, J. Path. & Bact. **34**:453, 1931.

A characteristic disease of guinea-pigs is caused by a hemolytic streptococcus indistinguishable in its cultural characters from *Streptococcus pyogenes*. *S. pyogenes* isolated from cases of human disease does not produce the lesions characteristic of the guinea-pig disease. The guinea-pig streptococcus retains its infective power during at least ten months' cultivation on ordinary laboratory mediums. Acute and chronic lesions may exist together in the same animal. The lesions are not confined to the lymphatic glands, as has been claimed, but occur in the lung, liver, spleen, mammary gland and muscles.

AUTHOR'S SUMMARY.

LESIONS OF THE ADRENALS OF RABBITS CAUSED BY HERPES VIRUS. W. SMITH, J. Path. & Bact. **34**:493, 1931.

A particular strain of herpes virus was found capable of producing specific lesions characterized especially by cell necrosis in the adrenal glands of rabbits. The lesions only occasionally followed the introduction of virus into the skin, testes or brain, whereas intravenous inoculation of an adequate dose was found to cause them in nearly every case. The lesions occurred in both the cortex and the medulla; their nature is briefly described. Typical herpetic intranuclear inclusion bodies were present, chiefly in the early stages of the disease; they were more numerous in the medullary than in the cortical foci. The injection of herpes immune serum with the virus prevented the formation of adrenal lesions in all cases. Normal serum and vaccinia immune serum had no such protective action. The production of adrenal lesions is not peculiar to one strain of virus, but with some strains preliminary modification by testicular passage may be necessary.

AUTHOR'S SUMMARY.

A STRAIN OF *BACILLUS AERTRYCKE* WITH UNUSUAL EPIDEMIC CHARACTERS. W. W. C. TOPLEY, M. GREENWOOD and J. WILSON, *J. Path. & Bact.* **34**: 523, 1931.

Certain strains of *Bacillus aertrycke*, of which the strain G. S. W. is an example, produce a widespread infection of mice exposed to contact with infected mice, without causing a severe and fatal epidemic. In so doing they produce a relative increase in the average resistance of the surviving mice, which is, or may be, significantly greater than that resulting from vaccination, either because a latent infection is a more efficient immunizing process than the inoculation of killed bacteria, or because the effect of the elimination by death of the innately more susceptible mice is added to the effect of active immunization. Probably both play a part. If such strains exist in nature, or are evolved during the course of epidemic spread, they may well play a part in determining the course of events, especially during a prolonged endemic-epidemic prevalence. It seems reasonable to suppose that bacterial variants that arise during cultivation in the laboratory may arise under natural conditions, but the proof that such variants play a rôle in epidemic disease awaits their demonstration in the field or their isolation during an experimental epidemic initiated with a strain possessing the more usual characters. The labor and expense involved in testing the epidemic potency of a single bacterial strain make it impossible to examine in this way large numbers of strains drawn at random from an infected herd. It is therefore unlikely that variants of the G. S. W. type would be detected, unless they formed a considerable proportion of the total number of strains of their own bacterial species distributed among the animal hosts.

AUTHORS' SUMMARY.

ENZOOTIC HEPATITIS OR RIFT VALLEY FEVER. R. DAUBNEY and J. R. HUDSON, *J. Path. & Bact.* **34**:545, 1931.

A virus disease affecting sheep, cattle, goats and man is reported from the Rift Valley of Kenya Colony. The virus causes a mortality of over 95 per cent in very young lambs, abortions and a moderate mortality in ewes and cows, and a transient fever accompanied by severe pains in the regions of the joints in man. In susceptible domesticated animals, the characteristic lesion is found in the liver. Histologically, this lesion is a focal necrosis, bearing a striking resemblance to the Councilman lesions of yellow fever. The virus passes regularly through Chamberland filters up to the grade L 11 and occasionally through the L 13 candle. The disease is not contagious, and there is evidence to suggest that it is transmitted by a mosquito, probably *Taeniarhynchus brevipalpis*. It is suggested that the disease falls into a natural group with yellow fever and dengue in man.

AUTHORS' SUMMARY.

GELATINOTHORAX. ROBERT ANGUS HUNTER, *Tubercle* **12**:204, 1931.

The author uses a 1:2,000 dilution of acriflavine in a 5 per cent gelatin solution adjusted to  $pH$  8 and finds this a suitable medium for intrapleural injection. The medium, being aqueous, mixes in even suspension with the effusion. It inhibits, and is lethal to, tubercle bacilli and other pyogenic organisms. The solution is used in amounts of from 20 to 600 cc.

H. J. CORPER.

THE EFFECT OF ENVIRONMENT ON THE VIRULENCE OF BCG. K. T. SASANO and E. M. MEDLAR, *Tubercle* **12**:214, 1931.

The pathogenicity of BCG depends largely on their environment. When grown on a bile glycerin potato medium, they are relatively innocuous, even when given in large doses. When they have been continuously cultured for a short period on Sauton normal rabbit serum medium, they become virulent.

H. J. CORPER.

EPIDEMIOLOGY AND PATHOLOGY OF TUBERCULOSIS IN INDIA. A. C. UKIL, *Tubercle* **12**:244, 1931.

The author presents rather incomplete figures on tuberculosis in India and reports on 1,000 consecutive necropsies performed in Calcutta during the last thirteen years, as well as on additional cases from two medical colleges. The post-mortem examinations revealed essentially continuous degenerative processes, as well as cavities, but nothing definite and conclusive. The explanation of massive infection in an imperfectly immunized soil interprets most of the lesions found in India. The secondary bacterial flora are believed to be richer and more varied than in Europe. The influence of climate and sunlight is discussed.

H. J. CORPER.

LEPROSY, A FILTRABLE VIRUS. J. MARKIANOS, *Ann. Inst. Pasteur* **46**:291, 1931.

The comparative study of rat leprosy leads me to propose as regards human leprosy (1) that in Hansen's disease there exists a virus capable of passing through the Chamberland filter, (2) that this filtrable virus reproduces the disease, (3) that young subjects are more susceptible to the ultravirus than adults and (4) that the granular stage forms a part of the evolution of the ultravirus toward the bacillary stage and of the bacillary stage toward the ultravirus. AUTHOR'S CONCLUSIONS.

TRANSMISSION OF SUBACUTE NEPHRITIS. JEAN TROISIER, *Ann. Inst. Pasteur* **46**:296, 1931.

Among the types of human nephritis there exists a virulent type, characterized clinically by a syndrome of subacute nephritis (albuminuria, cylindruria, edema, dyspnea, arterial hypertension, cantering rhythm, neuroretinitis, azotemia and anemia, without hypercholesteremia) and characterized anatomically by tubal nephritis with diffuse histologic lesions, subacute, epithelial (alteration of the tubules, granular and leukocytic cylinders) and interstitial (infiltration by lymphocytes).

This disease of man may be transmitted to the monkey (*Cercopithecus fuliginosus*) by inoculating the peritoneal cavity of this animal with an emulsion of renal parenchyma. The death of the monkey is thus induced in a week, with indications of acute nephritis (increased azotemia, albuminuria, cylindruria) and diffuse renal lesions (changes in the tubules, granular and leukocytic cylinders in the renal parenchyma).

AUTHOR'S CONCLUSIONS.

## EXPERIMENTAL STUDY OF SPIROCHAETA HISPANICUM (VAR. MACROCANUM).

H. VELU, L. BALOZET and G. ZOTTNER, *Arch. Inst. Pasteur de Tunis* **20**: 21, 1931.

Studies of the virulence of *Spirocheta hispanicum* in various vertebrate hosts revealed a number of silent infections. The results suggest that the rat is an important, but not the only, reservoir of the virus. Spirochetes were demonstrated in brain tissues after 447 days (and less) with the guinea-pig and after 6 months with the rat. Evidence indicated that this was a latent infection rather than a specific neurotrophic action. Splenectomy or blocking of the reticulo-endothelial system did not affect the course of the infection. In mixed infection with *Trypanosoma macrocanum*, the spirochetosis did not affect the trypanosomiasis, but there was some stimulation in the reverse direction.

M. S. MARSHALL.

A CASE OF CHRONIC PLAGUE LASTING SEVENTEEN MONTHS. PAUL DURAND, *Arch. Inst. Pasteur de Tunis* **20**:77, 1931.

Several cases are discussed, one in detail, in which virulent plague bacilli were demonstrated in persons who were outwardly free from the typical disease. A survival of the organisms in glands seventeen months after the initial infection was

observed in a boy, aged 14, who recovered following large doses of anti-plague serum. The glands were swollen and inflamed, and inoculation of guinea-pigs as well as nine of ten cultures gave positive results. The author brings out (1) that insect-borne plague may arise from cases in human beings as well as from those in rats, (2) that human infection may at times be endogenous, and (3) that influenza, measles or other predisposing causes may open the road for infections of the blood stream and complications with plague.

M. S. MARSHALL.

TOXIC SUBSTANCES PRODUCED BY STAPHYLOCOCCI. O. GENGOU, Arch. internat. de méd. expér. 6:211, 1931.

Experiments with rabbits are reported that confirm the previous statements of the author that staphylococci produce a substance capable of dissolving different cells in vitro and of producing toxic action in vivo. This substance is elaborated as much in vivo as in vitro and probably plays a definite rôle in staphylococcic infection. The secretion in vitro is not dependent on the degeneration, but on the normal activity of the micro-organisms.

J. N. PATTERSON.

THE BIOLOGY OF "STRONGYLOIDES STERCORALIS." H. E. PRÓES, Thesis, Bahia, 1930.

Dr. Próes describes an interesting fatal case of strongyloidosis in which "rhabditiform" larvae were discovered during the lifetime of the patient in the pleural effusion that was present on the right side. Their presence here was confirmed at autopsy, and identical larvae were found in the stools, in the pulmonary blood and in a small pericardial effusion. Dr. Próes' case is of special interest because the larvae were unusually widely disseminated, as they have so far been observed only in heavy infestations in animals. It is evident that the larvae in the pleura came from the lungs and those in the pericardium probably from the pleura by way of the lymphatics. It is also unusual to find the rhabditiform, instead of the filariform, larvae. The author concludes that a transformation of the rhabditiform into the filariform larvae is possible in the interior of the human body.

N. OPHÜLS.

## Immunology

THE SPECIFICITY OF SEROLOGICAL REACTIONS WITH SIMPLE CHEMICAL COMPOUNDS (INHIBITION REACTIONS). K. LANDSTEINER and J. VAN DER SCHEER, J. Exper. Med. 54:295, 1931.

Experiments are described that confirm the observation that the specificity of inhibitory reactions involving substituted aromatic acids is decidedly influenced by the position of the substituent. When antigens with specific groups of very simple constitution are used for the tests, inhibiting effects are obtained also with substances distantly related to those determining the reactivity of the antigens. On the other hand, if antigens are built up from protein and chemical compounds of somewhat higher complexity, the specificity of the inhibition reactions with synthetic crystallized substances is of the same order as that of the usual serum reactions; in other words, it is possible to distinguish such compounds by serologic tests as readily as proteins can be differentiated with the aid of precipitating serums.

AUTHORS' SUMMARY.

THE SPECIFIC CYTOTOXIC ACTION OF TUBERCULIN IN TISSUE CULTURE. J. D. ARONSON, J. Exper. Med. 54:387, 1931.

Tuberculins from the human and from the bovine type of tubercle bacilli inhibit the growth of cells from explants of bone marrow, spleen and testes of tuberculous guinea-pigs and are toxic for these cells, but have no effect on explants of the

same tissues from nontuberculous animals. "Tuberculins" from other acid-fast bacteria have no inhibitory or toxic action on explants of tissues from either tuberculous or nontuberculous guinea-pigs. Tuberculins from the avian, bovine and human types of tubercle bacilli, as well as "tuberculins" prepared from the Duval and from the Kedrowsky strains of *Mycobacterium leprae*, inhibit the growth of the cells of explants of the spleen and bone marrow of tuberculous fowls and are toxic for these cells, but have no effect on the explants from tissues of nontuberculous chickens. "Tuberculins" from other acid-fast bacteria have no effect on the growth of explants of tissues from tuberculous or from nontuberculous fowls. Tissue culture methods indicate that the sensitivity of tuberculous tissues to tuberculin is inherent in the cell, and that it cannot be passively transferred.

AUTHOR'S SUMMARY.

REFRACTORINESS TO THE SHWARTZMAN PHENOMENON CAUSED BY MOCCASIN VENOM. S. M. PECK and H. SOBOTKA, *J. Exper. Med.* **54**:407, 1931.

The majority of rabbits receiving intradermal, intraperitoneal or intravenous injections of moccasin venom became refractory to the development of the Shwartzman phenomenon. A period of incubation of about fourteen days was required for the resistance to develop. The incidence of refractory animals was inversely proportional, within limits, to the amount of toxin given intravenously to elicit the Shwartzman phenomenon. The intravenous route was the most efficacious in developing refractoriness. The refractory state was still present forty-four days after the primary injection of moccasin venom. Rattlesnake venom was not efficacious in inducing a refractory state. The refractory animals did not show a changed reaction to moccasin venom in the concentrations used. No circulating antibodies could be demonstrated to explain the refractory state. Antivenene had no effect on the course of the Shwartzman phenomenon.

AUTHORS' SUMMARY.

CHEMO-IMMUNOLOGICAL STUDIES ON CONJUGATED CARBOHYDRATE-PROTEINS. O. T. AVERY and W. F. GOEBEL, *J. Exper. Med.* **54**:431 and 437, 1931.

The p-amino and p-nitromonobenzyl ethers of the specific polysaccharide of type III *Pneumococcus* have been prepared. The diazonium ether of the specific polysaccharide has been coupled with serum globulin to yield a specific polysaccharide-protein complex, and this complex has been used for immunization.

Type-specific antipneumococcus immunity has been induced in rabbits by immunization with antigen prepared by combining a specific derivative of the capsular polysaccharide of type III *Pneumococcus* with globulin from horse serum. Rabbits immunized with this antigen acquire active immunity against infection with virulent type III pneumococci. The serums of the immune rabbits contain type-specific antibodies that precipitate the type III capsular polysaccharide, agglutinate type III pneumococci, and specifically protect mice against type III infection. The experimental data are discussed with reference to: the concurrence in the immune serums of type-specific antibodies for *Pneumococcus* and precipitins for horse globulin; the determining influence of the capsular polysaccharide on the specificity of the antigen as a whole; the unity of the type-specific precipitins, agglutinins and protective antibodies induced by a single component of *Pneumococcus* in chemical union with an unrelated, animal protein.

AUTHORS' SUMMARIES.

CHEMICAL AND IMMUNOLOGICAL STUDIES OF THE PNEUMOCOCCUS. A. WADSWORTH and R. BROWN, *J. Immunol.* **21**:255, 1931.

Until the ether-soluble material in the pneumococcus cell can be obtained in quantities sufficient for complete chemical and immunologic analyses, it is difficult to determine the significance of its various antigenic activities or its relationship

to other substances concerned in the immune reaction. Since these ether extracts appear to be free from protein, glycerol, sterol and carbohydrate, it is important to note the fixation of complement in the presence of antipneumococcus serums of all types and the lack of definite specificity in this activity. In vivo, no immunity was demonstrated against pneumococcus infection in mice inoculated with the ether extractive, and suspensions of dried pneumococci extracted with ether immunized mice to the same extent as suspensions of unextracted cells. It was not possible to demonstrate agglutinative, precipitative or complement-fixing activities in the serums of inoculated rabbits, although mice were protected against pneumococcus infection by such serums.

AUTHORS' SUMMARY.

HEMAGGLUTINATING PROPERTIES OF UREASE. M. HOTCHKISS and H. TAUBER, *J. Immunol.* **21**:287, 1931.

Crystalline urease in solution produced agglutination of the erythrocytes of the rabbit and of the mouse, but not of the sheep. In the tests in which high concentrations of urease were used, hemolysis occurred, complicating the results. This was subsequently controlled by the use of a buffer solution; then agglutination could be readily seen. After agglutination the urease was found to be active. Agglutination could not be demonstrated in the whole blood of rabbits dead after injection of urease. Crystalline urease represents a highly purified form of toxic hemagglutinin.

AUTHORS' SUMMARY.

EXTRACELLULAR MENINGOCOCCUS TOXINS. N. S. FERRY, J. F. NORTON and A. H. STEELE, *J. Immunol.* **21**:293, 1931.

Bouillon filtrates from young cultures of the four recognized Gordon types of the meningococcus contain extracellular toxins specific to the four types as well as a toxin common to all the types. Positive skin reactions have been obtained from meningococcus bouillon filtrates diluted as high as 1:1,500. When the four toxins are injected individually into animals, antitoxins develop specific to the types injected, as well as some antitoxin of the other three types. When all four toxins are injected at various times into animals antitoxins develop specific to the individual types. Meningococcus meningitis convalescent serums possess neutralizing properties apparently specific toward these soluble toxins. Control experiments with autolysates and mechanically produced cellular extracts from meningococci tend to identify further the toxins in the meningococcus bouillon filtrates as of extracellular origin.

AUTHORS' SUMMARY.

QUANTITATIVE STUDY OF COMPLEMENT FIXATION. A. WADSWORTH and E. and F. MALTANER, *J. Immunol.* **21**:313, 1931.

Although the values obtained with different complements varied, the relationship between the values derived from titration of different complements with different dilutions of the immune serum was constant under those conditions in which the course of the reactions was constant. The change in activity of the individual complements in the presence of the different dilutions of immune serum was directly proportional to the increase in the concentration of the serum. These constant relations made it possible to establish a unit value of complement activity based on the specific fixability of the complement and thus to determine the activity of any dilution of an immune serum consistently, with different complements.

AUTHORS' SUMMARY.

BRUCELLA ANTIBODIES IN HUMAN SERUM. J. W. MARTIN and J. T. MYERS, *J. Prev. Med.* **5**:243, 1931.

Brucella antibodies were found in serums from meat-packing employees as follows: in 15 per cent of the serums of one hundred employees who within

eighteen months had been on the company's sick-list with symptoms suggestive of undulant fever, and in 3 per cent of those of one hundred other employees. Brucella antibodies were found in 4.3 per cent of one thousand serums submitted for Wassermann tests. There was no appreciable difference in antibody incidence between Negroes and white persons. The three methods of examination employed—complement fixation, macroscopic agglutination and Huddleson's rapid macroscopic agglutination—gave parallel results and appeared to be equally accurate for laboratory diagnosis. Huddleson's method is very convenient. Either the liquid-air or the shaking-machine method of breaking the Brucella organisms for antigen is efficient. The optimum amount of serum to use in the complement-fixation test for undulant fever is 0.1 cc.

AUTHORS' SUMMARY.

THE SIGNIFICANCE OF AGGLUTININS IN NORMAL PERSONS. L. C. HAVENS and C. R. MAYFIELD, *J. Prev. Med.* 5:295, 1931.

We have described in this paper observations which show that agglutinins develop as a result of specific antigenic exposure under natural conditions, even without a clinical infection, and that in the absence of the antigenic opportunity such antibodies do not develop; that agglutinins rarely are found for infections which are known to have a low incidence, and that so-called "normal" agglutinins behave in the same manner as, and are in all respects indistinguishable from, specific antibodies. The clinical interpretation of a single observation is always a difficult matter. There seems to be no doubt that exposure to certain kinds of bacteria will start up again the reproduction of apparently unrelated antibodies such as have developed previously in response to an antigenic stimulus of a different nature; but this phenomenon, it seems to us, is not an example of nonspecific production of antibodies and does not invalidate our interpretation of "normal" agglutinins. It does, however, seriously interfere with interpretation of diagnostic agglutination tests. Such interpretation must always be qualified by the known chances of a possible antigenic opportunity in the past. The fact that there is no evidence or knowledge of an antigenic exposure does not preclude the possibility that this exposure has occurred. Examination by Krumwiede of 786 normal men in connection with a paratyphoid outbreak revealed 32 temporary carriers who showed no symptoms of infection. Ramsey found that yellow fever in Senegal is propagated in a mild, unrecognized and almost subclinical form and concluded that "the native population must constitute a vast but hidden reservoir of infection." The conclusion, therefore, seems justified that the demonstration of antibodies for a specific infection may be interpreted as the result of exposure to that infection at some time and in some manner. The further conclusion follows that the incidence of antibodies against a given infectious agent is one index of the prevalence of the infection in the region in which the study is made.

AUTHORS' SUMMARY.

USE OF PROTOZOA IN MEASURING THE NEUTRALIZING VALUE OF COBRA ANTISERUM. C. H. PHILPOTT, *Science* 74:157, 1931.

The strength of cobra antiserum is measured by placing paramecia in various mixtures of venom and antiserum and determining the least amount of antiserum required to protect paramecia from a given amount of venom. The results of ten titrations are shown.

AUTHOR'S SUMMARY.

IMMUNITY IN TRACHOMA. CHARLES NICOLLE and UGO LUMBROSO, *Arch. Inst. Pasteur de Tunis* 20:241, 1931.

Repeated intravenous inoculations with the Olitski strain of *Bacterium granulosis* did not procure immunity against a subsequent inoculation with trachoma



virus. Cross-protection tests as checks on potentially specific viruses or organisms seem a possibility, since the scar tissue of healed natural trachoma is protected against a virus very active on a new subject.

M. S. MARSHALL.

BACTERICIDAL EFFECTS OF TISSUES FROM HEALTHY AND IRRADIATED ANIMALS.  
G. M. ANTONIOLI, *Strahlentherapie* 41:496, 1931.

The organs of guinea-pigs that had been bled to death were ground with quartz powder under sterile conditions. Twice their weight of distilled water was added, and after shaking, the material was kept in the icebox for twenty-four hours. Two cubic centimeters of the supernatant fluid was then inoculated with staphylococci from a suspension of a twenty-four hour agar culture. After three, six, nine, fourteen or twenty-four hours' incubation agar plates were made. It was found that a more or less marked decrease in the number of colonies was present in comparison with that of the corresponding control plates. These results were, however, not constant, and great variations occurred. The liver seemed to possess the best bactericidal action, while that of the spleen, omentum and kidney was less marked. After repeated, weak roentgen irradiations of the animals an increase of the bacterial action of the different tissues was observed. An intensive roentgen irradiation of the animals resulted in a lowering of the bactericidal action of their organs. The difference in the bactericidal action of the various organs tested is explained by their unequal content of reticulo-endothelial tissue.

WILHELM C. HUEPER.

THE MORPHOLOGY OF THE PHENOMENA OF HYPERSENSITIVITY AND IMMUNITY.  
W. PAGEL, *Ztschr. f. d. ges. exper. Med.* 77:396, 1931.

The usual hypersensitivity of rabbits is not raised by tuberculous infection. Such hypersensitivity is not related to the diminution of precipitating antibody, since the phenomenon of Arthus also takes place in the absence of the antibody. In the case of histamine poisoning there is no difference between the normal and the tuberculous animals. The protection against anaphylactic shock by tuberculous infection is dependent chiefly on the formation of antibodies. The antigen in tuberculous guinea-pigs and that in normal guinea-pigs are not acted on in the same way. The injection of india ink and trypan blue does not weaken the phenomenon of Arthus or lower the titer of the precipitin. Although granulomatous and tubercle-like formations do not occur in connection with serum hypersensitiveness in the skin, yet in rabbits, as a result of intrarenal injections, such lesions are produced in the kidneys, where there results the picture of an "interstitial lymphoplasmocytic nephritis." The tuberculous granuloma may therefore be the tissue response of an antigen-antibody reaction, without the tubercle bacillus entering into the process. In cross-infections, in rabbits, with a human tubercle bacillus infection superimposed on a primary bovine tuberculous infection (and conversely), the secondary infecting agent is often quickly destroyed.

SANDER COHEN.

THE EVALUATION OF ORGAN EXTRACTS FOR THE WASSERMANN REACTION BY MEASURING THEIR TURBIDITY. J. ADAMSKI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 71:129, 1931.

The changes in the turbidity in a mixture of the extract and of an alcoholic solution of cholesterol were estimated for twenty-four hours with the instrument of Moll. Qualitative and quantitative evaluation was made possible by comparing with values for standard extracts. Different curves permitting comparative estimation were obtained for extracts according to their efficiency.

I. DAVIDSOHN.

NEW SEROLOGIC DIAGNOSIS OF TYPHOID AND PARATYPHOID BACILLI AND OF RELATED BACTERIA. K. AOKI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:138, 1931.

By using proper variants, absolutely specific agglutinating serums were produced for nine bacterial species. Nonspecific serums were produced for the same micro-organisms by using the nonspecific variants. Both types of serum are used for identification of the bacteria of this group. The specific serums identify the micro-organism; the nonspecific serums establish its relationship to the other species. The specific agglutination shows coarse floccules or granules; the non-specific, group agglutination shows fine floccules or granules.

I. DAVIDSOHN.

CONCERNING THE FACTORS M AND N OF LANDSTEINER AND LEVINE. M. AKUNE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:147, 1931.

When present alone, each of these factors is more sensitive than when they are together, as shown by agglutination experiments. Absorption experiments were found to be less conclusive. The use of serums with high titers is therefore recommended. No quantitative difference was observed in the sensitivity of factor M in the serums of the new-born infants and adults; its behavior in this respect is different from that of the A and B factors.

I. DAVIDSOHN.

EXPERIMENTAL STUDIES ON THE FRACTIONS OF THE LENS PROTEIN. H. HOFFMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:171, 1931.

The difficulties in production of lens antiserum are emphasized. Following the procedure of Woods and Burky, it was possible to separate fraction  $\alpha$ , but not fraction  $\beta$ . Precipitating serums were obtained only with the full antigen and with the suspensions of lenses. Such serums fixed complement with homologous as well as with heterologous lens suspensions. Antiserums produced with  $\alpha$ -crystallin of beef, hog and horse reacted in the complement-fixation test constantly and more specifically with the homologous antigen than with  $\beta$ -crystallin. Antiserums produced with  $\beta$ -crystallin showed no such specificity. Anaphylactic experiments confirmed the organ specificity of the lens and showed a certain degree of specificity for the  $\alpha$ -fraction.

I. DAVIDSOHN.

THE QUANTITATIVE RELATIONS IN LIPOID-ANTIBODY PRODUCTION FOLLOWING COMBINED IMMUNIZATION. J. VAN DER SCHEER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:190, 1931.

Large doses of Forssman antigen from horse kidney used in combination with hog serum had an inhibiting effect on the production of antisheep hemolysin in rabbits.

I. DAVIDSOHN.

SEROLOGIC STUDIES ON PUTREFACTION OF MEAT. FELIX SULMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:385, 1931.

Owing to their molecular structure, the simpler protein derivatives (amino-acids, putrefaction bases and urea derivatives) cannot act directly as antigens in the complement-fixation test. To overcome that difficulty they were added to the antigen-antibody complex of decaying meat and of a specific antiserum produced in rabbits with decaying meat, and according to the principle of Landsteiner's excess inhibition it was expected that if the substance tested was closely related to the antigen of putrefaction, the complement fixation would be interfered with. Of the thirty-one substances tested, none showed a relation to the antigen in question.

I. DAVIDSOHN.

IMMUNOLOGIC CLASSIFICATION OF *SALMONELLA PARATYPHI* (B. PARATYPHOSUS A) OF BRION AND KAYSER. R. MAESHIMA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:399, 1931.

An analysis of numerous strains of this species, with the help of agglutinations and absorptions, led to their separation into three subgroups, each with a specific agglutinating immune serum. However, all of them reacted with a serum that was specific for the entire species *Salmonella paratyphi*.

I. DAVIDSOHN.

FRACTION-SPECIFICITY AND SPECIES-SPECIFICITY OF SERUM PROTEINS. IVAN GYÖRFFY, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:428, 1931.

Horse serum was separated with various concentrations of ammonium sulphate into four fractions every one of which showed precipitogenic and anaphylactogenic properties. Common features were found in the precipitating serums produced by the first two fractions (obtained by 33 and 50 per cent ammonium sulphate saturation) and in their anaphylactogenic qualities; neither of the two reacted with fraction 4. The fraction displayed serologic relations with all others. It is concluded that there are only two serologically strictly fraction-specific serum proteins: albumin and globulin. The species of specificity as tested by precipitation with ox serum and its fractions was only relative.

I. DAVIDSOHN.

SERODIAGNOSIS OF SYPHILIS WITH THE ACETONE-INSOLUBLE LIPOID FRACTION OF KISS. ÖDÖN FISCHER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:441, 1931.

The extract of Kiss, which is the phosphatid fraction of beef heart (without the addition of cholesterol), proved a satisfactory antigen for the precipitation test, and in proper dilution also for the complement-fixation test. It is probable that the antigenic function does not rest with the phosphatids, but is due to some unknown substance present as an admixture.

I. DAVIDSOHN.

OLD AND YOUNG ANTIGENS AND ISO-ANTIBODIES OF OLD AGE. E. FRIEDBERGER and I. GURWITZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:453, 1931.

Young and fresh antigens (bacteria and red blood cells) were not more antigenic in vivo than old ones. Contrary to other reports, young rabbits did not develop isoprecipitins for the proteins of old rabbits when treated with their serum.

I. DAVIDSOHN.

## Tumors

HEREDITY OF SPONTANEOUS LEUKEMIA, PSEUDOLEUKEMIA, LYMPHOSARCOMA AND ALLIED DISEASES IN MICE. MAUD SLYE, *Am. J. Cancer* **15**:1361, 1931.

Slye affirms that "although data on human leukemias are few, they are in agreement with the facts demonstrated in her laboratory: first, that the leukemic diseases have an hereditary basis and tend to run in families; second, that they tend to occur in cancer strains."

B. M. FRIED.

INTRACRANIAL NEOPLASMA IN LOWER ANIMALS. MAUD SLYE, HARRIET F. HOLMS and H. GIDEON WELLS, *Am. J. Cancer* **15**:1387, 1931.

Tumor of the brain is rarely observed in lower animals of any species. In 11,188 mice of the Slye stock, only 3 cerebral neoplasms were found.

B. M. FRIED.

METASTATIC CARCINOMA SIMULATING HYPERPARATHYROIDISM. R. L. MASON and S. WARREN, *Am. J. Path.* 7:415, 1931.

A case in which there were widespread bone changes, hypercalcemia, negative calcium balance and thyroid tumor is presented. The roentgenologic appearance of the bones suggested metastatic carcinoma. Examination of parathyroid-like bodies removed at operation showed probable metastatic carcinoma from mammary cancer removed five years before.

AUTHORS' SUMMARY.

ADENOMA OF THE ISLANDS OF LANGERHANS WITH HYPOGLYCEMIA, SUCCESSFULLY REMOVED. N. A. WOMACK, W. B. GNAGI and E. A. GRAHAM, *J. A. M. A.* 97:831, 1931.

A man, aged 44, had hypoglycemic seizures, which were found to be due to an adenoma of the islands of Langerhans. A small tumor, about 0.5 cm. in diameter, was removed from the anterior surface of the pancreas at about the junction of the body and the tail. The tumor was made up of cells resembling island cells, yet differing from them in certain important details, especially in granular secretory antecedents. The mechanism of the production of apparent hyperinsulinemia remains obscure.

CARCINOMA IN OSTEOMYELITIC SINUS. E. B. BENEDICT, *Surg., Gynec. & Obst.* 53:1, 1931.

The author reports 12 cases of epidermoid cancer developing in the sinus of chronic osteomyelitis. These cases occurred in a series of 2,400 cases of osteomyelitis at the Massachusetts General Hospital. The malignant changes developed in 8 cases of osteomyelitis of the tibia, in spite of the fact that in the 2,400 cases the osteomyelitis was most common in the femur. In 3 of the cases, the carcinoma developed in the sinus of osteomyelitis of the bones of the foot, and in 1 case in the femur. A chronic discharging sinus was present for thirty years or more in all of the 12 cases, all of which occurred in elderly men. Biopsy was the most reliable adjunct to diagnosis.

C. G. WARNER.

PRIMARY CANCER OF THE THYMUS WITH PLURIGLANDULAR DISTURBANCE. O. LEYTON, H. M. TURNBULL and A. B. BRATTON, *J. Path. & Bact.* 34:635, 1931.

Two cases have been described in which carcinoma of the thymus appears to have been associated with pluriglandular disturbance. In a boy, aged 11, fatness of the face, conspicuous hypertrichosis of the face and prominence of the abdomen developed within a month. In a man, aged 31, increasing fatness of the neck and trunk and striae atrophicae on the abdomen developed during less than a year. During the same period, he suffered from night sweats, thirst and polyuria. Both patients, on admission to the hospital, were found to have diabetes mellitus. In both, at necropsy, a small cell medullary carcinoma of the thymus was associated with great hypertrophy of the cortices of the suprarenal bodies, functional hypertrophy of the thyroid gland, infiltration of the medullae of the suprarenal bodies by lymphocytes and plasma cells and fatty infiltration of the centers of the hepatic lobules. Duguid and Kennedy (1930) have described glycosuria, enlargement of the suprarenal bodies and colloid goiter in association with a small cell medullary carcinoma of the thymus in a woman aged 64.

AUTHORS' SUMMARY.

INNERVATION OF HUMAN TUMORS. H. OERTEL, H. NYE and B. THOMLINSON, *J. Path. & Bact.* 34:661, 1931.

The observations here recorded confirm the conclusions previously expressed, that mature and immature tissues of human tumors are innervated, and that this innervation applies to the blood vessels and also to the stroma and parenchyma

of the growing tumor. We base this conception on the close co-ordination of newly formed nerve fibers with the stroma and parenchyma in which they end, and to the cells and nuclei of which they are so intimately attached that a resemblance to normal innervation is definitely noticeable. In tumors, as in other tissue movements, the advance occurs as tissue entity, not in isolated parts.

AUTHORS' SUMMARY.

THE PRODUCTION OF EXPERIMENTAL TUMORS BY MEANS OF RADIOACTIVE SUBSTANCES. F. DAELS and R. BILTRIS, *Bull. Assoc. franç. p. l'étude du cancer* **20:32**, 1931.

The production of malignant tumors by the use of chemical substances is complicated in that the exact dose of the cancerigenic substance cannot be estimated. But with the use of stable radioactive substances the problem appears to be rather simple. This method has yielded positive results in experiments on white rats and mice. The authors give a brief résumé of successful experiments by previous investigators. They give in detail protocols of a number of their own experiments on the albino rat, the albino mouse and the guinea-pig. Ten sarcomas were produced in rats in a period of from eight to twenty-two months. Three sarcomas developed in mice in eight months, and one epidermoid cancer in ten months. It took from ten to twenty-five months to induce two sarcomas in guinea-pigs, and nearly two years to initiate epithelial blastomas of the biliary tract, the stomach and the kidney, respectively, in the same animal.

The authors emphasize that by the introduction of radioactive foci one obtains results that are superior to those obtained by previous methods in that a greater variety of tumors are produced, and also in that it is possible to inaugurate blastomas in different organs. The article is profusely illustrated.

B. M. FRIED.

TWO CASES OF CANCER OF THE BREAST IN THE MALE. DESSAINT and PLANTEVIN, *Bull. Assoc. franç. p. l'étude du cancer* **20:94**, 1931.

In one case, the epithelioma originated in a man aged 46; in the second case, the patient was 73 years old. The incidence, the etiology and the pathology of the disease in the male are discussed.

B. M. FRIED.

THE ACTION OF PROTEINS ON THE GROWTH OF CANCER GRAFTS. G. FAVILLI, SPERIMENTALE, *Arch. di biol.* **84:489**, 1930.

The stimulation of the reticulo-endothelial system by means of hypodermic injections of proteins favors the growth of grafts of mouse cancer and rat sarcoma in mice and rats, respectively. This is proved by the greater number of positive results and the more rapid evolution of the grafts compared with those in control animals. Such results stand in opposition to the presupposition that the stimulation of the reticulo-endothelial system and of its defensive powers checks the growth of grafts. An explanation of this fact is furnished by the development of grave splenic amyloidosis caused by the treatment with proteins.

G. PATRASSI.

A PRIMARY LEIOMYOPLASTIC SARCOMA OF THE SKIN. W. NEUMANN, *Centralbl. f. allg. Path. u. path. Anat.* **52:65**, 1931.

The tumor mass, 4 by 5 cm. and of at least two years' standing, was removed from the ankle of a woman 48 years old. Clinically, it had been diagnosed a fibroma, and macroscopically it resembled a desmoid or myoma. Microscopically, the origin of the tumor from the smooth muscle of the blood vessel coursing through it was evident. Although evidently malignant, the tumor had not formed metastases.

GEORGE RUKSTINAT.

NEURINOMA OF THE PIA MATER OF THE BRAIN. G. PATRASSI, *Centralbl. f. allg. Path. u. path. Anat.* **52**:209, 1931.

A meningeal tumor on the medial surface of the right occipital lobe of a woman 47 years old formed the basis for this report. The tumor was 6 by 7 by 5.5 cm., was found in the pia mater, and had no definite capsule but showed a thickening of its fascicular network near the periphery. The outer layers consisted of a fibrillar network rich in long nuclei, which imparted a bundle-like appearance to the mass in places. The network was not connective tissue, and in it were concentric arrangements of cells as in a pacinian corpuscle. The central fields had, instead of a fibrillar and cellular syncytium, coarse and compact strands of a homogeneous substance that reacted typically for connective tissue. Also here occurred masses of flattened and polyhedral epithelial-like cells. In the portions near the brain were retrogressive changes due to compression. In order to reconcile the various structures in the tumor with its location, the author agrees with Oberling that the pia may act to the brain as Schwann's sheath does to peripheral nerves. Thus a tumor arising from a meningoblast (a primitive cell of neuro-epithelial nature) might become epithelial, glial or sarcomatous. The author noted the resemblance, in some locations, of the pacinian corpuscle-like structures to the hypertrophy of Schwann's cells in progressive hypertrophic neuritis.

GEORGE RUKSTINAT.

RESPIRATION OF NORMAL AND TUMOR TISSUES. BRUNO KISCH, *München. med. Wchnschr.* **78**:1254, 1931.

The results of many experiments with twenty animals bearing the Jensen rat sarcoma and the Ehrlich mouse carcinoma, and of study of their tumors, livers, hearts and kidneys and the tissues of healthy control animals, lead to the conclusion that tumor tissues in contrast with all of the others have a marked stability or torpidity toward respiratory stimulants. Respiratory inhibition by a dearth of calcium salts does not occur in tumor tissues. Small doses of trivalent elements such as boron and aluminum do not act as respiratory stimulants in tumor tissues, although higher concentrations are toxic as in normal tissues.

AUTHOR'S SUMMARY.

THIRTY YEARS' STATISTICS FROM THE RIGA STADTKRANKENHAUS. *Ztschr. f. Krebsforsch.* **32**:280, 1930.

Among 14,893 autopsies performed at the Riga city hospital in the last thirty years, 2,083 showed the presence of malignant tumors. During the five year period from 1900 to 1904, 11.6 per cent of the males and 12.55 per cent of the females showed tumor, while in the corresponding period from 1925 to 1930 the percentages were 20.42 and 16.15 per cent, respectively. In Riga, as in most European cities, there has been a relative increase since the war in the groups of more advanced ages. In both sexes, cancer of the alimentary tract is becoming less frequent, except that there has been a threefold to fourfold increase of pancreatic cancer. Prostatic cancer, formerly relatively rare, has shown an increase in recent years. With uterine cancer there has been no change; with ovarian, a slight increase. Cancer of the lung has increased greatly in frequency; in 1929, it was ten times as frequent in men, and nine times in women, as in 1900. Tumor of the brain was found at autopsy twice as often in women as in men, and it appears to be increasing in frequency in the former sex, while becoming less common in the latter. As regards the lymphatic system, malignant growth here was commoner in males. During the thirty years there has been no evident change in the age distribution of cancer of the various organs.

H. E. EGGERS.

A MIXED CARCINOMA OF THE DUCTUS CHOLEDOCHUS. A. FEHR, *Ztschr. f. Krebsforsch.* **32**:367, 1930.

Fehr describes a cancer of the common bile duct that showed the presence of both glandular epithelium and keratinized stratified squamous epithelium. In both the primary tumor and its metastases, the two types were connected by such definitely intermediate forms as to leave little doubt of their development from a common source.

H. E. EGGERS.

TRAUMA AND TUMOR FORMATION. R. WERNER, *Ztschr. f. Krebsforsch.* **32**:599, 1930.

Werner discusses the relations of trauma, in the broadest sense of the word, to the causation of tumors. He points out the wide differences in the probability of this association between certain types of injury, as for instance that by x-rays, in which cancer is a not infrequent sequel, and mechanical trauma, in which it follows only occasionally. The latter relationship is the one he discusses most fully. He points out that an uninterrupted sequence of changes between injury and cancer is not essential, since at times the latter may appear at a site of previous injury or irritation even though, with removal of the causative element, there may have intervened a long period of apparently complete healing. The development of malignancy in these or allied circumstances may be rendered more probable by the action of concomitant factors, such as associated syphilis, prolonged or repeated action of the initial injurious factor, or the culminative effect of various agencies—to say nothing of what can only be termed a familial predisposition. As regards the usually accepted belief that a single trauma is more likely to be followed by sarcoma and long continued chronic irritation by carcinoma, he points out that exceptions to this rule are sufficiently frequent to make it impossible to predict with any certainty the type of malignancy that will follow injury of either sort. As an explanation of the mechanism by which injured tissues become malignant, he suggests that it is the result of a more or less gradual alteration in the cellular metabolism, brought about in conditions of acute trauma by consequent persistent "internal irritation"—an alteration from the normal oxidative metabolism to the glycolytic type studied principally by Warburg.

H. E. EGGERS.

CANCER OF THE LUNG IN THE MINERS OF JOACHIMSTHAL, CZECHO-SLOVAKIA. H. SIKL, *Ztschr. f. Krebsforsch.* **32**:609, 1930.

While the occupational incidence of cancer of the lung has been noted in the case of the Schneeberg miners for a considerable length of time, it is only recently that it was found by Löwy that miners of the Joachimsthal region were also subject to it. Since then the problem has become a matter of state investigation, with autopsies whenever possible. There have been ten autopsies among fifteen deaths in the last eighteen months, and in no less than eight of these cancer of the lung was found. Analysis of a portion of one of the diseased lungs failed to show the presence of any substance of presumable cancerigenic action, and Sikl suspects that the high radioactive emanative content of the air of the mine may be responsible.

H. E. EGGERS.

PRECANCEROUS CHANGES CAUSED BY THE X-RAYS. O. SCHÜRCH, *Ztschr. f. Krebsforsch.* **33**:1, 1930.

Schürch reports the results of studies of the cancerigenic action of the x-rays on the rabbit's ear. A long period, two years or more, and a prolonged and heavy dosage, short of single applications sufficiently dense to cause immediate necrosis, were necessary, but even with these precautions development of cancer was only occasional. Such animals as did not become cancerous showed changes

of somewhat varied types; in some, there appeared small, warty lesions, with perforation supervening later, with or without preceding atrophic changes; in others, the latter were the outstanding feature. When the warty growths were present, they continued to develop throughout the period of exposure, but did not become cancerous. In still other cases, there was early and extensive, but delimited, necrosis. In those lesions that later developed into cancer, the outstanding peculiarity, after atrophy and perforation, was the appearance of successive and recurring small erosions, at the sites of which usually, but not invariably, local hyperplasia and development of cancerous growth occurred. Cancer evidently did not occur as a regular stage of the inflammatory reaction, but only through a peculiar evolution of this; so that roentgen dermatitis is definitely precancerous only in the clinical sense; in it cancer is a secondary, nonessential change, and the morphologic changes of roentgen precancer in the rabbit are those of the secondary erosive lesions.

H. E. EGGERS.

PRECANCEROUS CHANGES. O. SCHÜRCH, *Ztschr. f. Krebsforsch.* **33**:35, 1930.

In a general discussion of the nature of precancerous change, to which he appends an exceptionally complete bibliography, Schürch takes the well grounded position that precancer does not so much represent a definite disease type as an intermediate stage between normal and cancerous tissue. Morphologically, this stage is represented by proliferative epithelial changes, atypically arranged and with abnormally formed cells and nuclei, accompanied by changes in the underlying connective tissue. But even these changes are not necessarily precancerous, as they are distinctly reversible. Just as important as the morphologic changes are the clinical features—the character of the irritation, the nature of the tissue, the heredity and the possible metabolic or endocrine dysfunction. Clinically, a sharp differentiation should be made between lesions that are actually cancerous, such as basal cell carcinoma or Paget's disease of the nipple, lesions that experience has shown are precancerous with some regularity, such as senile hyperkeratosis, papillary leukoplakia and lentigo maligna, and lesions that become cancerous only occasionally and exceptionally, such as lupus vulgaris.

H. E. EGGERS

DOUBLE TUMOR OF THE STOMACH. A. SCHUBACK, *Ztschr. f. Krebsforsch.* **33**:126, 1930.

There is reported a case of double tumor of the stomach—adenocarcinoma and lymphosarcoma—both of which were apparently primary there. The writer suggests as the probable explanation a primary carcinoma, with irritation of the lymphadenoid tissue to the point of malignant metaplasia by material absorbed from the first tumor; all this occurred in a person in whom both types of tissue were highly susceptible to malignant change.

H. E. EGGERS.

CHORIOCARCINOMA IN THE MALE SEX. C. G. AHLSTRÖM, *Acta path. et. microbiol. Scandinav.* **8**:213, 1931.

The discussion is based on three tumors: a retroperitoneal choriocarcinoma arising from a retained anlage; a combination in one testicular tumor of choriocarcinoma, large cell carcinoma and adenocarcinoma; an adenocarcinoma of the testis admixed with syncytial elements, in the metastasis of which were typical choriocarcinomatous formations. In spite of the morphologic similarity between choriocarcinoma in woman and that in man, the evidence at hand is not sufficient to establish a histogenetic identity. The following considerations speak against the identity of choriocarcinomas in the two sexes: Teratomatous choriocarcinoma occurs almost exclusively in the male sex, with localization in the testicle; the metastases are localized quite regularly in the retroperitoneal lymph nodes, and combination with carcinoma may occur, suggesting that choriocarcinoma in the male sex is a peculiar testicular tumor.



### Medicolegal Pathology

STRUCTURAL CHANGES IN THE BRAIN OF DOGS IN RAPID CARBON MONOXIDE ASPHYXIA. J. CHORNYAK and R. R. SAYERS, *Pub. Health Rep.* **46**:1523, 1931.

Chornyak and Sayers studied the cerebral changes produced in dogs by fatal exposures of twenty to thirty minutes to 0.6 per cent by volume of carbon monoxide in air. 1. The circulatory changes in the brain in rapid carbon monoxide asphyxia are characterized by dilatation, stasis, perivascular hemorrhage and edema. 2. Edema is diffuse and severe. It is both perineuronal and perivascular. 3. There is a marked difference in the susceptibility of the nerve cells to oxygen deprivation. The cells of the cortex, corpus striatum, dorsal motor nucleus of the vagus and the dorsal sensory areas of the medulla are the most sensitive. The nucleus ruber, nuclei of the oculomotor, trochlear, abducens and facial nerve and the large polygonal cells in the reticular formation of the medulla are the least susceptible. 4. There are two general types of degenerative changes in the nerve cells following asphyxia: (a) Some become shrunken and stain diffusely; (b) others show varying degrees of chromatolysis. 5. Carbon monoxide produces a diffuse degenerative change throughout the entire brain. 6. In this type of asphyxia the most serious effect appears to be edema of the dorsal motor nucleus of the vagus and the adjacent area in the medulla oblongata. c

THE LEGAL AND SOCIAL MEDICINE OF AUTOMOBILE ACCIDENTS. C. SIMONIN, *Ann. de méd. lég.* **11**:286, 1931.

The large majority of automobile accidents are due to physical or mental inefficiency of the persons concerned. The solution of the problem demands more efficacious physical and psychic examinations of prospective drivers, thorough and immediate factual and pathologico-anatomic investigation of all accidents by medical experts, and enlightened methods of educating both the motoring and the nonmotoring public.

E. M. BARTON.

FATAL POISONING BY SODIUM CHLORATE. C. STRYZYOWSKI, *Ann. de méd. lég.* **11**:528, 1931.

The demonstration of a chlorate in the organs after poisoning by that salt is rare, but it may generally be found in the urine, as in the case of a 65 year old man who swallowed from 20 to 30 grains (1.3 to 1.95 Gm.) of sodium chlorate by mistake. Death followed in less than twelve hours. Painstakingly controlled chemical examination demonstrated a chlorate in considerable amounts in the urine obtained at necropsy, and traces of it in the brain and kidneys. None was found in the stomach, liver or blood. The latter was brown from methemoglobin. The lethal dose of sodium chlorate is probably about 12 grains (0.78 Gm.).

E. M. BARTON.

SUBCUTANEOUS HEMORRHAGES IN BULLET WOUNDS. R. PIÉDELIEVRE and P. ÉTIENNE-MARTIN, *Ann. méd. lég.* **11**:569, 1931.

The circular zone of ecchymosis in the skin surrounding the orifice of entry of bullet wounds is not due, as formerly held, to trauma by the gun muzzle. It is present in wounds by bullets fired at a distance. In addition, experiments in firing shots through varnished rubber sheets have demonstrated cracks and defects in the varnish in patterns analogous to the zones of hypodermal hemorrhage seen microscopically, as well as grossly. Apparently a bullet entering the skin stretches it causing rupture of small blood vessels in the less supple subjacent tissues.

E. M. BARTON.

ACCIDENTAL FLUORIDE POISONING. J. SEDLMEYER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:228, 1931.

A detailed account is given of an accidental poisoning, first suspected of being a homicide, due to contamination of food (flour) with sodium silico fluoride ( $\text{Na}_2\text{SiF}_6$ ) with description of the chemical procedures employed in its detection.

E. L. MILOSLAVICH.

SUICIDAL FLUORIDE POISONING. J. SEDLMEYER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:234, 1931.

The author gives a brief description of a case involving a 21 year old woman who ingested an insect powder, with suicidal intent. Severe vomiting and diarrhea, intense convulsions with subnormal temperature and unconsciousness developed, leading to a rapid, fatal end. The chemical analysis of the remaining powder, as well as of the internal organs, proved the presence of sodium silico fluoride ( $\text{Na}_2\text{SiF}_6$ ). The mucous membrane of the stomach and of the upper part of the small intestines appeared swollen, hyperemic and partly covered with hemorrhages.

E. L. MILOSLAVICH.

### Technical

AN IMPROVED ANTIGEN FOR THE COMPLEMENT-FIXATION TEST IN YELLOW FEVER. MARTIN FROBISHER, JR., *Am. J. Hyg.* **14**:147, 1931.

The serum of persons or monkeys actively immunized to yellow fever will fix complement when mixed with saline extracts of the liver from monkeys that have died of yellow fever. A new procedure for the preparation of the antigen, made fat-free, is presented.

PAUL MERRELL.

PRESERVATIVE FOR SMALL BLOOD SAMPLES SENT THROUGH THE MAILS. A. R. ROSE and F. SCHALTNER, *J. Biol. Chem.* **92**:17, 1931.

A powdered, anhydrous mixture of sodium sulphate and sodium fluoride is recommended for the desiccation and preservation of small samples of blood. A series of samples preserved in this manner showed no significant change in sugar content after storage for intervals of from 0.25 to 1.5 years.

ARTHUR LOCKE.

HINTON, KAHN AND WASSERMANN TESTS IN DIABETES. H. F. ROOT and G. O. STUART, *New England J. Med.* **204**:1179, 1931.

The three tests were made simultaneously on 1,078 specimens of diabetic blood. There was disagreement in the results in 59 cases. Variations in the chemical composition of the blood did not cause false positive tests in any case. The Kahn test gave the largest number of doubtful results and the Hinton test the smallest.

THE PREPARATION AND STANDARDIZATION OF COLLOIDAL GOLD FOR THE LANGE TEST. J. PATTERSON, *Brit. J. Exper. Path.* **12**:143, 1931.

A method is described for the preparation of colloidal gold for use in the Lange test in which the reactions are more closely controlled and a more uniform product obtained. A single preliminary test can be carried out on a small sample to insure success of the bulk preparation, and the process itself is relatively immune to the presence of traces of impurities. A method for adjusting the reaction of the colloidal gold is also described, the standard being a solution of oxyhemoglobin rather than titration with saline solution.

C. E. CLIFTON.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Oct. 8, 1931*

LEILA CHARLTON KNOX, *President, in the Chair*

INFECTION WITH A SO FAR UNDETERMINED PARASITE (Lantern Slide Demonstration). ALFRED PLAUT.

By a curious coincidence, two instances of infection with a parasite unknown to the author and never seen by many authorities to whom the slides were submitted came to observation within the course of a few weeks. They are reported in the hope that other observations of a similar kind may be made, or perhaps may already be on record.

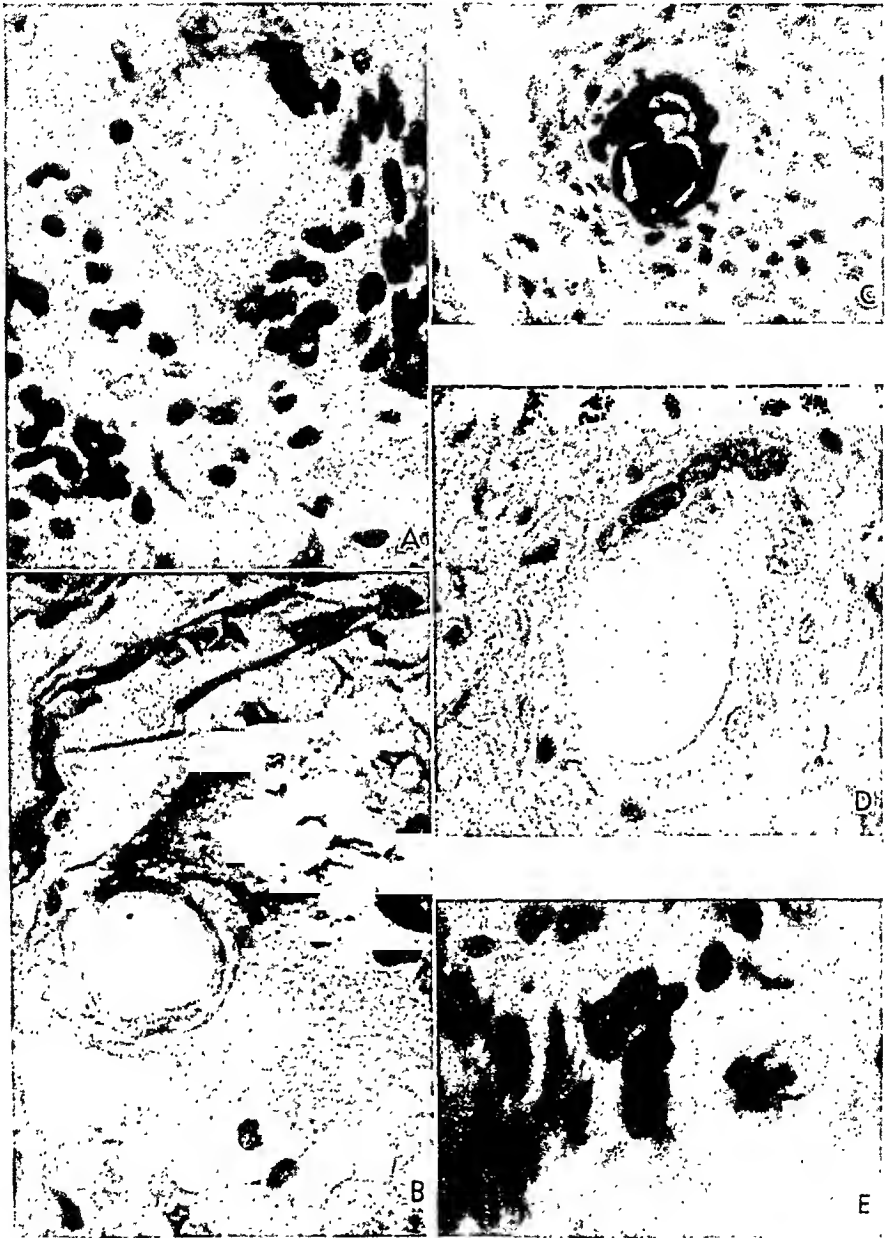
CASE 1.—A white man, 38 years old, a resident of New York, had been in good health up to two years ago, when nausea and repeated vomiting with inability to retain food caused him to lose 25 pounds (11.3 Kg.) within a few weeks. He felt weak and had no appetite. He forced himself to eat and tried to restrain the vomiting. He was a man of asthenic type, emaciated and pale, but not acutely ill. There was a tenderness on pressure in the right upper quadrant of the abdomen. There was a suggestion of a mass in that area.

A diagnosis of carcinoma of the stomach was made, and a resection performed. At operation the stomach appeared normal from the outside; the great omentum contained numerous, large lymph nodes. The interior of the stomach was polypoid. After the resection and the gastrojejunostomy, the patient recovered and was discharged twenty days after operation. He went to a convalescent home for several months, and then resumed his office work. He had gained 30 pounds (13.6 Kg.), and he retained his weight, but still remained underweight. Later examinations revealed persistent eosinophilia (about 10 per cent) in his blood, with otherwise normal blood findings. X-ray pictures of the chest showed old and recent tuberculous lesions.

The gross specimen consisted of a piece of stomach measuring about 10 by 9 cm. A piece of omentum 15 cm. wide and 5 cm. long was attached, and contained lymph nodes measuring up to 3 cm. long. They were homogeneous and fairly soft, with a light brownish, granular cut surface. Smaller nodes were found in adhesions between the stomach and the omentum. The serosa otherwise was smooth. The gastric wall was normal in thickness in most of the specimen, but one end was considerably thickened, up to a little more than 1 cm. The tissue, however, did not appear firm. The condition of the mucosa at this end could be judged, since an incision had been made previously. The thickening was partly due to swelling of the mucosa and partly to hypertrophy of the muscle coat. Sections were taken from the thickened part and the very thin part of the specimen.

Microscopically, the thickening appeared to be due mainly to hypertrophy of the muscle. The mucosa was widely infiltrated with epithelioid cell tubercles, the picture being such that nobody at first glance would hesitate to diagnose tuberculosis. The pictures very much resembled the universal sclerosing, tuberculous, large-celled hyperplasia described by Mylius and Schuermann. The difference, however, was that in their sections only small, calcified bodies in giant cells were observed, while in our material distinct parasites could be found. These parasites at first resembled yeasts. They were, however, considerably larger. In the celloidin-paraffin section, the ovoid space from which a parasite had fallen out measured 44 microns (fig., D). In the paraffin sections, the measurements varied

between 28 and 34 microns. Few well preserved parasites could be found; perhaps none of them showed its original shape. They had a distinct shell, which occasionally showed a double contour (fig., *B*), and they had a round central body which, with calcification, became deep blue in hematoxylin; in some obviously noncalcified individuals, it was moderately blue (fig., *C*). The character of this



An undetermined parasite: *A*, giant cell containing ovoid cavity in which are broken masses representing the parasite; *B*, parasite the shell of which has a double contour; *C*, the round central body of the parasite, stained deep blue with hematoxylin; *D*, ovoid space from which a parasite has fallen out; *E*, a shamrock formation.

round mass could not be determined. Occasionally, a shamrock formation (fig., *E*) was found, with what appeared to be three nuclei in a partly subdivided, perhaps cytoplasmic mass. In most of the giant cells, the ovoid cavity contained only irregular, more or less broken masses (fig., *A*). The calcified individuals gave a positive Kossa reaction; otherwise, no special staining was successful. The Gram stain was negative; the parasites were not acid-fast. The lymph nodes from the omentum gave the same picture. This description applies also to the sections from the second case.

**CASE 2.**—A Russian woman, about 50 years of age, who was otherwise in good health, complained of heaviness in the eyelids which had increased within the last few months. In both eyelids, flat, somewhat crescent-shaped, firm infiltrations could be palpated. At operation on one eye, the infiltration was seen to reach deep into the orbit. The infiltration could be shelled out easily from between the tarsus and the skin of the eyelid. It was firm and on the cut surface grayish. The microscopie picture was as described.

From the material of the other eye, cultures were made on Sabouraud and other mediums, which had been especially adjusted for the growth of yeast. All the cultures remained sterile. Small pieces of the tissue a few minutes after operation were implanted into subcutaneous pockets in a dog, a rabbit, a guinea-pig, a rat and a mouse. The mouse died. All the other animals remained healthy for several months; the small wounds healed without reaction.

The x-ray picture of the chest of this woman revealed mediastinal swellings, which may or may not have been due to a similar granulomatous process.

#### AN ANALYSIS OF THE BLOOD PICTURE IN ONE HUNDRED CASES OF CANCER. MAURICE MORRISON (by invitation).

One hundred consecutive cases of cancer were studied hematologically. The bleeding time was less than one minute in two thirds of the cases. In only one instance, a case of cervical lymphosarcoma, it was increased (four minutes). The clot retraction time was normal.

The tourniquet test was almost always negative. The platelets varied from 150,000 to 400,000 in 95 per cent of the cases. Thrombocytopenia was uncommon. Thrombocytosis, on the other hand, was far more frequent.

The coagulation time was found to be within normal limits or slightly reduced.

The hemoglobin presented two extremes. Low readings, found in half the cases, were most frequently seen in those of gastro-intestinal cancer, the blood in cases of gastric involvement yielding an average of 38 per cent hemoglobin, and in cases of carcinoma of the colon, an average of 50 per cent. Higher readings were found in cases of pulmonary and mammary cancer, the hemoglobin averaging 67 per cent in the former and 69 per cent in the latter. In half the cases, a marked disparity was present in the relation between the hemoglobin and the red cell count.

In the study of anemia in malignant conditions, it was found expedient to employ the term erythrocytopenia to indicate a low red cell count, and the term hemoglobinopenia to indicate a low hemoglobin value. Cases in which there were lesions in the stomach yielded the lowest number of red cells (average 3,690,000), whereas those in which there were lesions in the colon, lung, breast and pancreas, and sarcomas, yielded the higher numbers, the average range being from 4,000,000 to 5,000,000 red cells per cubic millimeter. Higher red cell and hemoglobin values were found in cases with involvement of the left side of the colon than in those with involvement of the right side.

**Color Index.**—The majority of the cases showed a low color index, although in seventeen it was high.

**Red Cells.**—The most outstanding feature was anochromasia, which was present in fifty-two cases.

**Leukocytosis.**—This was present in 61 per cent of the cases. Two terms are used that require definition. "Asegmentopenia" may be defined as the absence of

a concomitant increase of band forms accompanying leukocytosis. This was observed in 85 per cent of the cases presenting leukocytosis. "Aneosinopenia" may be defined as a condition in which there is a persistence of eosinophils in the presence of a neutrophilic leukocytosis. Ninety-five per cent of the cases presenting neutrophilic leukocytosis were associated with aneosinopenia.

Twenty-one cases presented leukopenia, with a count below 8,000. Relative lymphocytosis was present in five of these. Metastases were present in 57 per cent of the cases presenting leukopenia.

*Types of Blood Pictures in Malignant Conditions.*—It was found that the blood picture presented in cases of malignant growth was of five types: (1) stimulative, (2) irritative or leukemoid, (3) infiltrative or dysplastic, (4) destructive or myelophthitic, and (5) indeterminate.

**Stimulative:** This type of picture, which was present in 55 per cent of our series, consists mainly of slight leukocytosis (from 10,000 to 12,000), moderate neutrophilia (from 75 to 80 per cent), asegmentopenia and no significant myeloid changes. Leukocytosis and neutrophilia run parallel. Aneosinopenia is usually present. If there are any changes in the lymphoid elements, there is usually an increase in the large variety. Erythrocytic changes are insignificant. This picture reflects a physiologic change in the bone marrow, which is mainly one of hyperplasia, due to stimulation. A hypothetic neoplastic toxin produced in the primary or metastatic focus may be responsible for this picture.

**Irritative, Leukemoid, Slight Osteoplasia:** This type of picture, which was present in 16 per cent of the cases, consists essentially of moderate leukocytosis (12,000 or more), and moderate neutrophilia (80 per cent or over). Asegmentophilia is usually present. The leukocytosis may be disproportionate to the neutrophilia; neutropenia may even occur. Asegmentopenia may sometimes occur; aneosinopenia is usually present. There may be an occasional myelocyte or metamyelocyte. There are no changes in the lymphoid elements; erythrocytic changes may consist of an increased reticulation or anochromasia. This picture may result from microscopic metastasis in the red marrow areas which may be detectable on roentgen examination, or the result of slight osteoplastic changes.

**Infiltrative, Dysplastic or Moderate Osteoplasia:** This type was found in 9 per cent of the cases. Essentially, the blood picture presents slight leukopenia (from 5,000 to 8,000), slight neutropenia (from 50 to 60 per cent) and relative lymphocytosis (from 30 to 40 per cent). If neutrophilia is present, there is usually a combination with the irritative, leukemoid, slight osteoplastic type. Asegmentopenia is usually absent, but may be present. Asegmentophilia is usually present, but may be absent. There may be present myelocytes and metamyelocytes. Should there be a relative lymphocytosis, there is a shift to the left; that is, the large lymphocytes are increased. Erythrocytes usually show moderate changes, as manifested by hypochromasia, anochromasia, polychromasia and occasional normoblasts. Pathologically, the bone marrow may be the seat of microscopic metastasis or of a moderate degree of osteoplasia. The metastasis may or may not be discernible in the x-ray picture.

**Destructive, Myelophthitic, Marked Osteoplasia:** This type of picture was present in 7 per cent of the cases. Essentially, the blood findings are as follows: moderate leukopenia (below 5,000), moderate neutropenia (below 50) and moderate relative lymphocytosis (over 40). Asegmentopenia is usually absent; asegmentophilia is usually present; myelocytes, metamyelocytes and myeloblasts may be present. If relative lymphocytosis is present, there is a shift of the lymphocytes to the left. Erythrocytic changes may be manifested by the presence of normoblasts, reticulocytosis and polychromasia. Pathologically, there may be macroscopic metastasis in the bone marrow or the presence of a moderate degree of osteoplasia.

**Indeterminate:** In this picture the criteria outlined are indefinite or insufficient for accurate interpretation. It was present in 13 per cent of the cases.

It is of interest to know whether a study of the blood can be of aid in early diagnosis, early enough to be of benefit to the patient. One may readily conclude

that the only cases in which this can be of aid is in those presenting the stimulative type of blood picture, before the onset of metastasis. For purposes of ultimate prognosis and treatment, the cases presenting the stimulative type of blood picture may be divided into two subgroups: (1) those without metastasis, and (2) those with metastasis. Sixty per cent of the cases that present the stimulative type of blood picture have no demonstrable metastasis. Theoretically, therefore, it should be possible to diagnose a malignant condition in almost one third of the cases at a time when metastasis is clinically absent.

#### DISCUSSION

MAX LEDERER: Dr. Morrison was prompted to make this study by two considerations. The first was the unsatisfactory condition of the literature regarding the blood in malignant conditions. There are contradictory statements in the literature, which are based on studies of small numbers of cases. He wished to see if in the blood pictures in an unselected group of 100 consecutive cases there was anything that might point toward a diagnosis of malignant growth, and serve perhaps either to exclude the condition or to corroborate the suspicion in the mind of the clinician. As a matter of fact, when put to the test, Dr. Morrison was able to arrive at more than a suspicion of malignancy in some doubtful cases in which the clinician was unable to make a diagnosis, and subsequent events showed that the cases were malignant, which bears out the assertion that a careful hematologic study in some instances may lead to a diagnosis of cancer.

There is one striking point, namely, the presence of leukocytosis, with the absence of band forms. This is not an unusual finding, and this one fact more than any other may strengthen a suspicion of malignancy. The absence of eosinophilia (Dr. Morrison used a term that is a rather peculiar one—aneosinophilia—but it is the only one that he could find to express his meaning) strengthens that suspicion still more. I think he has made a valuable contribution, however, from another point of view, and that is in attempting to divide the blood pictures into various groups according to types. I believe these represent changes in the bone marrow in the various stages of malignancy. Whether they are due to the presence of a hypothetic toxin or to the direct mechanical metastases we do not know. A great deal has been written on this subject, and so we do not want to speculate on it, but I feel that these five pictures do not represent different phases of the blood picture in malignancy, but rather different stages in the development of the disease, with perhaps an indication as to its severity.

#### EXTRAGENITAL CHORIONEPITHELIOMA IN A MAN. A. R. KANTROWITZ.

Necropsy disclosed in a man, aged 22, a teratoma of the anterior mediastinum containing chorionepitheliomatous elements. The tumor invaded the superior vena cava and studded both lungs with chorionepitheliomatous metastases. Careful gross examination revealed no metastases in other organs or lymph nodes. The genital tract (testes, vas deferens, seminal vesicles and prostate) showed no tumor nodules. The testes were sectioned in 2 mm. blocks, and slides were made from each block. Examination revealed no tumor nodules. Microscopic examination of the tumor revealed teratomatous and chorionepitheliomatous elements. Only chorionepithelioma was found in the pulmonary metastases. The testes showed no neoplastic elements. Marked interstitial cell hyperplasia was seen. These observations refute the contention of Prym and Oberndorfer, the latter writing "dass beim Mann das Chorionepitheliom immer mit Keimdrüesengeschwülsten in Zusammenhang stehen muss." The Aschheim-Zondek test was positive, both in the urine and in the extracts of the tumor tissue.

#### DISCUSSION

LEILA CHARLTON KNOX: In reference to the tumor that was reported by Dr. Lambert and myself, I reviewed the report of the autopsy for a definite statement about the testes. It is unfortunate that there is no definite record on this point.

The finding of a "healed tumor nodule" would not seem to me a contribution to the subject, since one must find actual tumor cells to establish a diagnosis.

ROBERT T. FRANK (by invitation): Dr. Kantrowitz asked me to discuss his case, and I do so with greater pleasure because I recall that the first presentation that I made in my youth was before this society twenty-six years ago, on this very subject. At that time, the discussion centered mainly on the morphologic characteristics of these growths; that is, the first report that was made of this type of tumor was by Malassez and Monod in 1878. They called it "angioblastic sarcoma," recognizing its intimate relationship with the blood vessels. In 1902, Wlassow recognized its resemblance to chorionic elements, both normal and abnormal, as we see them in hydatid moles, and Schlagenhauser spoke of their being formed on a teratomatous basis. I am confining what I have to say to teratomatous testicular tumors and their appearance in other organs, as well as primary chorionepithelioma of the mediastinum. As I said, at that time, the morphology was the only thing of interest, as the biologic method did not allow of testing. Today the matter stands on a different basis. With the various biologic tests available, we are more interested in determining whether the biologic reactions produced by these tumors are the same as those found in the female. The biologic tests in question are the prepituitary reaction first described by Aschheim-Zondek, in which we have three degrees to be considered: reaction 1, which is not specific for pregnancy but means simply that follicular growth is induced in the ovary, and reactions 2 and 3 of Aschheim-Zondek, which are specific for pregnancy. They show themselves macroscopically as ovulation with hemorrhage in the follicle and luteinization of the ovary in these immature animals. That is definitely specific. The recent work of Heide, Fels and Mathias, which was performed on the urine and blood in such a case of testicular chorionepithelioma, showed a definite pregnancy reaction. The other reaction is a demonstration of the female sex hormone in the blood and urine in these persons to a point higher than is found in males, because as most of you know it is occasionally found in small quantity in the male. In cases in which the tests have been successful, they have corresponded to the biologic reaction found in the chorionepithelioma of the female, that is, an exaggeration of the pregnancy reaction in both the male and the female.

Strange to say, we have had occasion at Mount Sinai Hospital to see three chorionepitheliomas in the male within a period of three or four weeks. One was observed only clinically in the sense that roentgen examination and biopsy determined scattered tumors in the lung of the patient. The presence of gynecomastia, a strong positive Aschheim-Zondek reaction and a very strong female sex hormone reaction strengthened the diagnosis. The second case was that of a chorionepithelioma of the testis removed with metastases, in which the same reactions were found. The third case was that presented here, in which a strong Aschheim-Zondek reaction was shown and in which while the female sex hormone test on the urine was unsatisfactory, because the quantity obtained post mortem was insufficient, an extract of the tumor tissue gave the female sex hormone reaction.

These biologic reactions may seem to the uninformed only curious phenomena, but they have an extremely valuable diagnostic and prognostic value. In these cases, during the presence of the growth, the reaction is positive; after removal of the tumor, it becomes negative, and several observations are on record of how, after a shorter or longer period had elapsed before clinical signs were present, the return of a positive reaction demonstrated the presence of growing metastases.

One further word: I think the criteria of Prym are a trifle too strict. I do not for a moment say that the testicle should not be carefully examined, but in cases in which there is a circumscribed three-layered teratoma in the mediastinum, I think it may be definitely stated that the primary origin of the chorionepithelioma is at that site.



PAUL KLEMPERER: I must agree with Dr. Frank that the criteria of Prym are a bit strict regarding the existence of a primary extragenital chorionepithelioma in the male. However, we have to face the fact that in the majority of these cases in which extragenital chorionepithelioma was diagnosed, the testicles were not examined sufficiently, and therefore there has been so far no definite proof that Prym's contention is not correct. In this case, the testicles were cut in very thin slices, and many sections of each slice were examined microscopically.

In regard to the fact that a tumor, particularly chorionepithelioma, may heal, I would like to point out that chorionepithelioma in the female may disappear. I remember a case of this kind in which a large tumor of the lung was excised and proved to be a chorionepithelioma. Later on the patient came to autopsy, and the uterus was examined carefully; no evidence of chorionepithelioma could be found.

We must be glad that this case was reported, because it proves definitely the existence of an extragenital chorionepithelioma in the male.

The possibility that a teratoma may metastasize with all its structures must be borne in mind. I have seen cases in which the metastases contained also mesodermal tissue. Of course, the chorionepitheliomatous part is usually the most malignant portion and produces the most widespread metastases.

#### FOREIGN BODY GRANULOMA DUE TO SPORES OF LYCOPodium. WILLIAM ANTOPOL.

Six cases of foreign body granuloma were presented. These patients had undergone a previous operation. On investigation it was found that the spores of *Lycopodium* had been used as a dusting powder at the initial operative procedure. These spores correspond to the foreign bodies found in the lesions.

#### DISCUSSION

ALFRED PLAUT: Dr. Antopol's demonstration touches a problem in which I have been interested for a long time. Foreign bodies and foreign body reactions become very frequent when one's attention is centered on them. A few examples of such lesions collected during the last few years may be demonstrated here by lantern slides.

The picture most frequently encountered is that of suture material within giant cells. The suture material is easily recognized by its optical characteristics. It is often found because it is so highly refractive. Stewart once stated that by examining sections of tissue in the polariscope one can find some foreign bodies that otherwise escape detection.

In the surface of the ovary of a woman who never had had any operation we see this very small, highly refractive foreign body with an intense inflammatory reaction around it. The character and the origin of this foreign body remain unknown.

During the autopsy on a man who at the age of 41 had died of prostatic carcinoma, a hyperemic lymph node was removed from the axillary fat. We see a piece of plant tissue which was situated within the lymph node without giving rise to any tissue reaction. Since this man had been a cigar worker, we can understand how the plant tissue came into his lymph stream.

The peritoneal foreign body reaction for obvious reasons is seen most frequently in the serosa of the appendix. It may appear as a small nodule consisting of more or less hyalinized layers of connective tissue with plant cells in the center. The patient from whose appendix this section was prepared had had typhoid fever thirty-six years before. It may be that through a perforated typhoid ulcer intestinal contents escaped.

Another slide demonstrates a piece of small intestine with plant fragments in the serosa. In this instance, as in many others, no history of abdominal operation, abdominal injury or manifest perforation of the digestive tract could be elicited.

Another section from the same patient contains large areas of structureless material similar to wood.

The calcified mass, measuring 20 by 18 microns, that we see in this slide can be recognized as being of plant character. In this instance, also, no evidence of the origin of the plant tissue could be found.

Only two weeks ago, in the course of a gynecologic operation, a small tumor of the serosa of the intestine caught the eye of the surgeon. This nodule also consists of connective tissue with plant seeds in the center. After the material had been dissolved in antiformin, many stone cells could be found.

Very peculiar pictures result from the presence of calcified round bodies similar to psammoma bodies in the peritoneum. They may, as Kaufmann stated, result from Schmorl's nodules after pregnancy. They may belong to the still unexplained disease of peritonitis arenosa. In our specimen, the psammoma bodies are found with small adenomatous tumor masses of unknown origin. The ovaries in this patient were normal.

Finally, to come nearer to Dr. Antopol's demonstration, I am showing small, round, distinct bodies that can be found very often in all kinds of specimens. They are plant spores similar to the spores of *Lycopodium*, only smaller and more regularly round. When material is handled and left open on the work bench, plant spores may fall into it from the air. Afterward, when the sections are carefully examined, because of an interest in eggs of parasites, these spores may easily deceive one.

The problem of how intestinal contents can escape so frequently without giving any clinical symptoms still awaits solution. In material from different sources I have an astonishingly large percentage from patients who either were born in Russia or have lived in southern countries.

PAUL KLEMPERER: I am surprised that Dr. Plaut did not show us one foreign body reaction that we see rather frequently, namely, that in the fallopian tubes. This is puzzling and leads sometimes to the diagnosis of tuberculosis. I think it is due in some cases to insufflation of the tubes.

ALFRED PLAUT: I was in the Woman's Hospital five years and never saw one.

WILLIAM ANTOPOL (closing the discussion): It might be of interest to note that in the last case in which *Lycopodium* granuloma had complicated tuberculous adenitis, there was a further complicating factor, in that there were crystals within the giant cells. Morphologically, these resembled magnesium silicate crystals of talcum. No attempt was made to identify these chemically.

## Book Reviews

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**A System of Bacteriology in Relation to Medicine.** Medical Research Council. Volume 6: Immunity. Pp. 537. Volume 8: Fungi, Streptothricaceae, Spirochaetes, Normal Flora, Swine Erysipelas. Pp. 390. Volume 9: Technical Methods, General Index. Pp. 363. Cloth. Price, per volume, 1 pound, 1 shilling, net; postage extra. London: His Majesty's Stationery Office. 1930. (May be obtained from the British Library of Information, 5 East Forty-Fifth Street, New York.)

The other six volumes of this system have been reviewed in the ARCHIVES (10:986 [Dec.] 1930; 11:333 [Feb.] 1931). The first review describes the general plan and scope of the work. Volume 6 is devoted mainly to immunity. There is a chapter on the relation of bacteria to disease and one on chemotherapy. The various parts of immunity are discussed by writers who are actively interested in the subject about which they write. The total result is a good and useful summary of the knowledge of immunity, probably the most comprehensive at present available in the English language. Not all the authors escape the temptation to discuss the problems and results of their own work so fully that a well rounded presentation is not achieved. Volume 8 contains articles on the pathogenic fungi, the spirochetes, the leptospira, the normal bacterial flora of the human body and swine erysipelas. The articles on syphilis and yaws fail to consider adequately the observations of medical officers of the American navy and army on the relationship between these diseases. Sporotrichosis is dismissed with a few words without mention of the identity of American and French sporotrichosis. And *Sporotrichum schenckii*, the causative agent of sporotrichosis, is designated erroneously as *Sporotrichum beurmanii* (for a full discussion of this matter of names see Davis, D. J.: University of Wisconsin Studies in Science, 1921, no. 2, p. 104). Volume 9 contains twenty-two articles on important topics in bacteriologic technic by authors selected because of special fitness for their respective tasks. No effort has been made to detail all the methods used for a particular purpose. The methods described are those that the authors of the articles have found to be reliable by personal experience, no matter whether original with the authors or not. The volume consequently gives a summary of the most advanced British technic. Probably not all the special methods of technic here described are of general interest at this time. The article on the breeding, maintenance and manipulation of laboratory animals contains much of practical value, but the methods of securing human material for bacteriologic and allied purposes and of making human tests with bacteriologic and related substances does not receive separate consideration. Volume 9 contains the general subject index. There is no author's index. As stated in an earlier review, there is no index in the separate volumes; the general index, which appears to be well arranged, compact and adequate, is the master key to all parts of the system. In conclusion, "A System of Bacteriology" is an important addition to the literature of microbiology and medicine. It gives a well considered and comprehensive summary, compressed within reasonable volume, of the knowledge of microbiology in its medical relations after it has filtered through the British bacteriologic mind. The Medical Research Council has conducted a difficult and complicated enterprise to a successful result.

**Cancer: Its Origin, Its Development and Its Self-Perpetuation.** By Willy Meyer, Consulting Surgeon to the Lenox Hill and Postgraduate Hospitals, New York Infirmary for Women and Children, etc.; Emeritus Professor of Surgery, New York Post-Graduate Medical School. Cloth. Price, \$7.50. Pp. 427. New York: Paul B. Hoeber, Inc., 1931.

In this volume Willy Meyer presents an elaborate and detailed theory of the origin and nature of cancer. He makes full recognition of the concurrent operation

in the causation of malignant neoplastic disease of local and constitutional factors, and it is in regard to the latter that his theory is most fully developed. This he regards as fundamentally a matter of imbalance of salt, with an increase in the body of potassium salts in replacement of those of calcium. He regards such a substitution as at times resulting from, and at times causing, irregularities of the sympathetic nervous system, with a disturbance of the normal sympathetic-parasympathetic balance, an associated upset of normal endocrine relations and a greater disturbance of the imbalance of salt, the whole constituting a vicious cycle that may be initiated at any point in the circuit. With the development of this imbalance, the body cells become hydropic, with a resultant impairment of the healing capacity.

In this condition, which he recognizes as nonspecific for cancer, locally irritated or injured body cells do not regenerate normally, and in the local circumstances of inferior tissues and insufficient supply of blood, there is further enhancement of the imbalance of salt and of alkalosis. In addition, the local process would result in impairment of the permeability of the tissues about the focus, even though the cells about to become cancerous might themselves be abnormally permeable as a result of breaking over of the surface film from a water-in-lipoid emulsion to one of lipoid-in-water. With the liberation of necrohormones from such cells as may have succumbed, the local retention of these in undue concentration and their ready absorption by the cells destined to become cancerous, an active process of cell division is initiated and is perpetuated by the spontaneous death, with a fresh liberation of necrohormones, of some of the now cancerous cells.

Intriguing as Meyer's theory may be, it should be fully recognized that it is largely speculative. While it offers an explanation, which may or may not be correct, of the possibilities in the way of predisposition to cancer, it accounts less satisfactorily for the local phases of the generation of cancer, for while the hypothesis of necrohormones may suffice to explain temporarily excessive cellular proliferation, it is difficult, particularly in view of the evidence derived from the experimental study of cancer, to conceive of the indefinite prolongation of such overgrowth by that mechanism. His theory takes almost no account of what would appear to be one of the most important developments of the modern study of cancer, the abnormal carbohydrate metabolism of the cancer cell, as elaborated by Warburg and his co-workers.

## Books Received

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INTRACRANIAL PYOGENIC DISEASES. A PATHOLOGICAL AND CLINICAL STUDY OF THE PATHWAYS OF INFECTION FROM THE FACE, THE NASAL AND PARANASAL AIR-CAVITIES. By A. Logan Turner, M.D., LL.D. (Edin.), Hon. F.R.C.P. (Edin.), F.R.S.E., Fellow of the Royal College of Surgeons of Edinburgh, and Consulting Surgeon, Ear and Throat Department, Royal Infirmary of Edinburgh, and F. Esmond Reynolds, M.D. (Edin.), D.T.M. & H. (Camb.), M.R.C.P. (Edin.), Superintendent of the Laboratory of the Scottish Asylums' Pathological Scheme, and Lecturer on Neuro-Pathology, University of Edinburgh. Price, 12 shillings, 6 pence. Pp. 271, with 82 illustrations, 21 in color. Edinburgh: Oliver & Boyd, 1931.

ASSOCIATION FRANÇAISE POUR L'ÉTUDE DU CANCER. ATLAS DU CANCER. Neuvième et dixième fascicules. LES TUMEURS DES CENTRES NERVEUX ET DES NERFS PÉRIPHÉRIQUES. Par Gustave Roussy et Charles Oberling, Fondation Henri de Rothschild. Paper. Paris: Félix Alcan, 1931.

ANNALS OF THE PICKETT-THOMSON RESEARCH LABORATORY. Volume 6. THE PATHOGENIC STREPTOCOCCI. THE RÔLE OF THE STREPTOCOCCI IN SCARLET FEVER. By David Thomson, O.B.E., Ch.B. (Edin.), D.P.H. (Camb.), Hon. Director, Pickett-Thomson Research Laboratory, St. Paul's Hospital, London, and Robert Thomson, M.B., Ch.B. (Edin.), Pathologist to the Pickett-Thomson Research Laboratory. Price, \$10. Pp. 470. Baltimore: Williams & Wilkins Company, 1930.

OSLER AND OTHER PAPERS. By William Sydney Thayer, M.D., LL.D., Dr.Hon., Sc.D., F.R.C.P., Ire.Hon., Professor Emeritus of Medicine at the Johns Hopkins University. Price, \$3.50. Pp. 386. Baltimore: The Johns Hopkins Press, 1931.

THE THOMSEN HEMAGGLUTINATION PHENOMENON. PRODUCTION OF A SPECIFIC RECEPTOR QUALITY IN RED CORPUSCLES BY BACTERIAL ACTIVITY. By V. Friedenreich. Paper. Pp. 138. Copenhagen: Levín & Munksgaard, 1930.

L'APPAREIL CARTILAGINEUX EN OTO-RHINO-LARYNGOLOGIE. Par J. Terracol, J. Turchini et H. Harant. Monographies Oto-Rhino-Laryngologie Internationales, No. 23. Paper. Pp. 216. Paris: Les Presses Universitaires de France, 1931.

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## ELLIPTICAL HUMAN ERYTHROCYTES

REPORT OF TWO CASES \*

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The red blood cells of mammals, with the exception of one family, are circular in outline. Those of the lower vertebrates, with the exception of one family, are elliptical or oval. The one exception among the mammals is a terrestrial family, the Camelidae, the red cells of which are elliptical. The one exception among the lower vertebrates is a marine family, the Cyclostomata, the red cells of which are round. Individual variation among the cells is unusual; Ponder<sup>1</sup> said of the Mammalia: "The uniformity of shape met with in the red cells is very striking and in well made preparations scarcely any deformed cells will be seen."

The erythrocytes of man, aside from the poikilocytes seen in anemia, ordinarily present the form of circular, biconcave disks, whether examined in the counting chamber, in fresh blood under a coverslip or in dried films. Günther,<sup>2</sup> however, called attention to the fact that in dry films of human blood an occasional elliptical cell is not infrequently seen.

There are on record a few cases in man in which as large a proportion as 95 per cent of the erythrocytes have been found to be oval or elliptical instead of round. It is the object in this paper to report two instances of this anomaly and to present a report of what the writers believe to be the first autopsy in such a case.

The first case of elliptical human erythrocytes was reported by Dresbach<sup>3</sup> in 1904 as occurring in a healthy young student whose red blood cell count was 5,000,000 and whose hemoglobin was up to standard. Ewald, in writing to Dresbach about this case, which was the subject of considerable correspondence at the time, said that he thought that a

\* Submitted for publication, Aug. 25, 1931.

\* From the Edward Hines, Jr., Hospital.

1. Ponder, Eric: The Erythrocyte and the Action of Simple Hemolysins, Edinburgh, Oliver & Boyd, 1924.

2. Günther, Hans: *Folia haemat.* **35**:383, 1928.

3. Dresbach, Melvin: *Science* **19**:469, 1904.

similar observation had been made at Königsberg some twenty or thirty years before, but no record of the case could be found.<sup>4</sup> Eleven cases were reported in the next twenty-four years: two by Bishop,<sup>5</sup> one by Sydenstricker,<sup>6</sup> two by Huck and Bigelow,<sup>7</sup> five by van den Bergh<sup>8</sup> and one by Bernhardt.<sup>9</sup> The number of known cases was doubled in 1929 by Hunter and Adams,<sup>10</sup> who discovered twelve cases in three generations of a family of Dutch origin living in Oregon and Montana. Lawrence<sup>11</sup> recently reported four cases. Eight cases that have not been recorded in the literature are known to us from personal correspondence, two of them observed by Jaffe,<sup>12</sup> two by Sydenstricker<sup>12</sup> and four by van den Bergh.<sup>12</sup> We are also permitted to refer here and elsewhere in our paper to a forthcoming article by Cheney<sup>12</sup> in which he will report fourteen cases in a family group comprising forty-one persons.

Of the fifty-two instances we have thus collected, including our own and Cheney's, thirty-four were found on investigating the families of the persons concerned in the previously discovered cases. We may say, then, that the anomaly has been discovered eighteen times in twenty-seven years—or in twenty-nine years if it is remembered that while Dresbach made his report in 1904, he discovered the case in 1902—and that it has been reported by eleven observers, including Cheney, whose paper is yet to appear, and ourselves. Of the fifty-two cases, twenty-eight occurred in males and twenty-four in females; forty-six of the persons concerned were white, five were black and one was a mulatto; the ages were from 3 years to 64 years; all four blood groups were represented.

#### DIFFERENTIAL DIAGNOSIS

The first case reported was that of a mulatto, and for this reason several of the earlier authors have been at some pains to exclude sickle

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4. Flint, Ewing, Ehrlich, Ewald and Arneth, cited by Dresbach, *Melvin: Science* **21**:473, 1905.

5. Bishop, F. W.: *Arch. Int. Med.* **14**:388, 1914.

6. Sydenstricker, V. P.: *J. A. M. A.* **81**:113, 1923.

7. Huck, J. G., and Bigelow, R. M.: *Bull. Johns Hopkins Hosp.* **34**:390, 1923.

8. van den Bergh, A. A. H.: *Arch. f. Verdauungskr.* **43**:65, 1928; *Deutsche med. Wchnschr.* **54**:1244, 1928.

9. Bernhardt, Herman: *Deutsche med. Wchnschr.* **54**:987, 1928.

10. Hunter, W. C., and Adams, R. B.: *Ann. Int. Med.* **2**:1162, 1929.

11. Lawrence, J. S.: *Am. J. M. Sc.* **181**:240, 1931.

12. Jaffe, R. B.; Sydenstricker, V. P.; van den Bergh, A. A. H., and Cheney, Garnet: Personal communication.

cell anemia, remembering Herrick's article on this subject,<sup>13</sup> which appeared in 1910. In a few instances pernicious anemia has been considered, but only to be dismissed. In the cases that we have here enumerated, neither of these conditions, nor any other, seems to have offered any difficulty, and the point will not be dwelt on further. In well marked cases, in persons in apparent health, as most of them have been, carefully made films have sufficed for the recognition of the condition. It is evident, however, from what Hunter and Adams found, that cases may easily be missed if slides show only a small percentage of elliptical cells and no clue of heredity is at hand; in seven of eleven persons presenting the anomaly among eighteen relatives whom they examined in their case, they noted that the unusual cells were "few" or "very few."<sup>14</sup>

#### ASSOCIATED DISEASE

The associated conditions have ranged from malaise to carcinoma, and treatment, when required, has varied from the simplest medication to appendectomy, in Bishop's case, and splenectomy for hemolytic jaundice, in one of van den Bergh's. In no case has any connection been established between the associated disease and the unusual shape of the erythrocytes, and in no case has treatment changed the shape of the erythrocytes.

#### HEREDITY

The hereditary nature of the anomaly, suspected by Dresbach and suggested by Bishop's two patients, a man and his sister, has been fully established by Hunter and Adams' large group and by Cheney's. Both sexes have transmitted it; a generation may be skipped, as shown by Bernhardt's case in one whose father and mother had normal erythrocytes.

In five of the original eighteen observations, fourteen related persons were investigated without the discovery of an additional case (Dresbach, Sydenstricker, Bernhardt and ourselves). In six of the eighteen, it was possible to investigate a considerably larger number of relatives, and among them, as we have said, thirty-four additional cases were found (Bishop, Huck and Bigelow, van den Bergh, Hunter and Adams, Lawrence, Cheney). In the remaining seven of the eighteen, no family search was reported (van den Bergh, Jaffe, Sydenstricker, Lawrence).

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13. Herrick, J. B.: Arch. Int. Med. 6:517, 1910.

14. It may be, as van den Bergh surmised and as Günther's observation suggests, that the condition is not so rare, after all.



Four of the thirty-four cases just referred to have been discovered recently by A. A. H. van den Bergh of Utrecht,<sup>15</sup> who succeeded in finding, scattered all over the Netherlands, eighty relatives of A. Kl., who, together with his descendants in America, comprise the group of twelve whose cases were reported by Hunter and Adams.<sup>10</sup> Van den Bergh found that four of the descendants of a sister of A. Kl. have the same peculiarity of elliptical erythrocytes; two are male and two are females; among them all four blood groups are represented.

#### NOMENCLATURE

So far no name has been assigned to the anomaly. In the *Quarterly Cumulative Index Medicus* (9: 419, 1931), we find "Erythrocytes, elliptical: See Anemia, sickle-cell," to which we may reasonably object, since these conditions are not related save as heredity is a factor in both. "Ovalocytosis" has been suggested, parenthetically, by Bernhardt,<sup>9</sup> and van den Bergh also has used this term but, likewise, only in parenthesis as a subtitle in writing of his latest discovered cases.

The objections to the term ovalocytosis are obvious: Not only is the word a hybrid, but it fails to indicate the erythrocyte as the cell concerned. The term ovalerythrocytosis or that of ovalerythropoiesis might be accepted, in spite of being hybrids, if it were not that the one implies an increase in the number of red cells, which is no part of the picture, while the other implies some knowledge of the process of the production of the anomalous cells, concerning which nothing is known. Since at present the condition is known only as an unexplained and unrelated morphologic peculiarity of the red blood corpuscles, it seems best to speak merely of oval or elliptical erythrocytes until such time as, happily, further knowledge may suggest a better word. "Elliptical" seems to us, from examination of our slides and from measurements made by others, somewhat the better word; it is the word used by the first two writers on the subject.

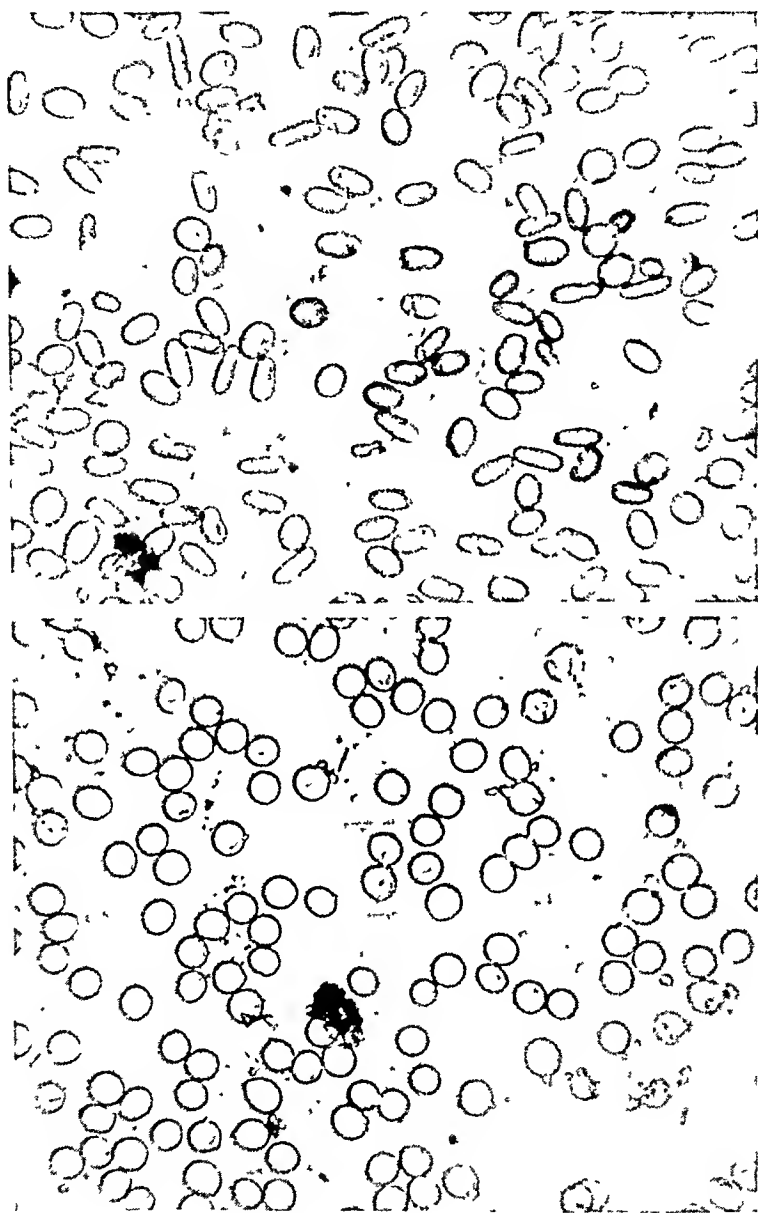
#### REPORT OF CASES

CASE 1.—John C., 36 years old, a colored truck driver, was admitted to the Edward Hines, Jr., Hospital, with a diagnosis of severe nephritis. His family history was unimportant. He had had measles, whooping cough and typhoid fever as a child, and gonorrhea and syphilis while he was in the army. For syphilis, he had received approximately forty injections of arsphenamine and seven of mercury. In January, 1929, he spent three weeks at the Red Cross Hospital, Louisville, Ky., because of chronic nephritis and heart trouble. He then returned to his home and worked until April when his symptoms returned. Thereafter he stayed at home until June 2, when he was admitted to this hospital.

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15. van den Bergh has made a short communication on the subject to the Royal Academy of Science at Amsterdam, and his paper may be expected to appear soon. See footnote 12.

On admission, he was somewhat stuporous, but rational and able to answer questions intelligently. He became progressively worse; edema of the ankles and coarse râles at the bases of the lung appeared. On June 29, Cheyne-Stokes' breathing was present. On July 12, the patient died.



The upper photomicrograph shows oval red cells (John C.); the lower, round red cells of normal blood; Wright's stain. The two films were made on the opposite ends of the same slide, by the same person, by the same technic and at the same time.

The clinical diagnosis was: severe interstitial nephritis, with uremia; severe arterial hypertension; chronic myocarditis; aortitis, and albuminuric retinitis.

There appeared to be nothing unusual about the case until an examination of the blood was made by one of us, when it was seen that by far the greater number of the erythrocytes were elliptical instead of round.

*Laboratory Findings.*—During the forty days that the patient was in the hospital, the red cell count varied from 3,500,000 to 4,000,000, with a color index never less than 0.9 and never greater than 1. The ellipticity of the red corpuscles was constant. The total white cell and the differential white cell counts were normal. The reticulocyte count on one occasion was 8 per cent. The coagulation time was normal. The blood was of group A. The fragility test will be referred to later. The Wassermann test of the blood gave a negative result; the Kahn test, "one plus." Analysis of the blood showed: total nonprotein nitrogen, 41.3 mg.; urea nitrogen, 16 mg.; uric acid, 5 mg., per hundred cubic centimeters of whole blood. The van den Bergh reaction, direct and indirect, was normal. The specific gravity of the urine was from 1.010 to 1.020; a few casts were present, with a moderate number of white blood cells, an occasional red cell and albumin, urobilin and urobilinogen. The test for renal function, phenol-sulphonphthalein injected intramuscularly, showed 5 per cent elimination in the first hour and 5 per cent in the second hour, a total of 10 per cent.

*Autopsy.*—Autopsy was performed twelve hours after death, the body remaining in the icebox meanwhile, not embalmed. The external appearance was that of a well developed, very black Negro of slender build, about 35 years old. There was moderate emaciation. The feet and ankles pitted on pressure. The hair of the head was abundant and in short tightly kinked curls. There were the normal amount and normal distribution of hair on the abdomen.

The pupils were normal. The teeth were large, white, even and unusually perfect. The face was long, oval and narrow. The cheek bones were rather prominent and the nose aquiline. There were no scars on the body, except an old scar from an operation in the groin. It was particularly noted that there were no scars or ulcers on the legs or feet.

The lungs did not meet. There was about 50 cc. of fluid in the left part of the chest and about 100 cc. in the right part. That in the right part was turbid and contained fibrinous flakes, which adhered to the visceral pleura. The left lung was somewhat compressed by the fluid in the pleural cavity and still more by the greatly enlarged heart. The lung was heavy and very wet from the contained frothy fluid. Numerous recent infarcts were present in both lobes. A few small calcified nodules were found in the upper lobe. The right lung was like the left, including infarcts, but without tuberculous nodules.

The apex of the heart was at the seventh interspace. The surface was dark, and the vessels were deeply congested. The pericardium contained a slight excess of clear fluid. Petechial hemorrhages were seen in both the inner and outer surfaces of the sac. The valves were normal. Some large antemortem clots were present. The aorta, in its first part, showed numerous yellow points, but no large plaques. The coronary arteries were normal. The heart weighed 610 Gm.

About 100 cc. of clear fluid was present in the abdomen. The stomach and the intestines, including the appendix, were apparently normal. The retroperitoneal glands were not enlarged.

On the anterior surface of the right lobe of the liver, there was a cruciform depression, the two lines of the depression being each about 2 cm. long. On section, the hepatic tissue had a nutmeg appearance. The liver weighed 1,450 Gm.

The gallbladder, the pancreas and the suprarenal glands were normal.

The spleen was small, firm and dark purple; the malpighian bodies were indistinct. The organ weighed 150 Gm.

The kidneys were small, brick red on section and granular looking. The capsule stripped with difficulty, leaving a granular surface.

The sternum, when cut into, showed a rather dry, pinkish, cancellous structure with little marrow. The ribs showed red marrow of normal appearance. In the left femur, the marrow, instead of being the usual yellow color and of thick consistency, was reddish purple and semifluid or lymphoid.

*Anatomic Diagnosis.*—The anatomic diagnosis was: cardiac hypertrophy and dilatation; infarction of the lung; pleurisy with effusion; old, healed pulmonary tuberculosis; chronic nephritis; chronic passive congestion of the liver, and an anomaly of the marrow of the femur.

*Microscopic Examination.*—Smears from the cardiac blood and from the spleen showed a predominance of oval-shaped erythrocytes. In the cardiac muscle, there was a notable increase of fibrous tissue, and elliptical erythrocytes were seen in the arterioles. The liver presented a connective tissue increase and parenchymatous changes incident to chronic passive congestion. In the kidney were marked interstitial increase of fibrous tissue, sclerosis of the blood vessels, particularly of the arterioles, and atrophy of many glomeruli. In the spleen, the connective tissue was increased, particularly about the blood vessels, and elliptical red cells were seen in the vessels. In the suprarenal glands, the blood vessels were widely dilated and full of blood, and wherever the erythrocytes were sufficiently separated, it was seen that most of them were oval instead of round. The marrow from the femurs revealed nothing significant, except an occasional oval erythrocyte. The young nucleated erythrocytes were round and in no way unusual in appearance.<sup>16</sup>

Three brothers, a sister and a maternal uncle of the patient were seen and all found to have normal erythrocytes. The blood group in one of the brothers was determined; it was group A.

CASE 2.—J. S., 26 years old, a colored porter, had previously been in the Edward Hines, Jr., Hospital because of tricuspid disease, but at that time no note was made of any peculiarity of his erythrocytes. When readmitted on Aug. 17, 1929, he was suffering from heart failure, which increased until his death, on Feb. 28, 1930. Examination of blood film showed a high percentage of elliptical erythrocytes. The presence of this condition was confirmed by many subsequent slides and other preparations that were as convincing as those in our first case.

Autopsy showed adhesions between the pericardium, the left pleura and the posterior wall of the chest and slight nodular thickening of the edges of the mitral leaflets, the valve being of normal size. The tricuspid opening was enlarged, measuring 14 cm.

There was an excess of fluid in the pericardial sac, with fluid in the right pleural and abdominal cavities.

An 8 year old son of the patient was found to have normal erythrocytes. The blood of both the father and the son belonged to group "O."

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16. Even in the lower vertebrates, the young erythrocytes are round, the oval form being acquired only as the cell approaches maturity. See Maximow, Alexander A.: *A Textbook of Histology*, Philadelphia, W. B. Saunders Company, 1930, p. 61.

## EXPERIMENTS AND COMMENT

Certain of our experiments have been done by all others who have had the opportunity to study similar cases. Some were designed to throw further light on the condition, and so far as we know, have not been done before. Unless otherwise indicated, the experiments described were done in the case of John C.

*Experiment 1.*—It was necessary to determine, first of all, if faulty technic was responsible for the unusual appearance of the erythrocytes. This was done by making many preparations from day to day with all possible care and controlling them by similar preparations of normal blood made at the same time. The result was that the more perfect the technic the more convincing was the evidence that the cells were truly elliptical, and that the less the manipulation the greater the percentage of such forms. In well made slides, the long axes of the oval cells lay in all directions and not predominantly in the direction of the smear. Most of the cells, in addition to being oval, were plainly biconcave. Deliberately faulty technic always operated to reduce the percentage of ellipses. We found, for example, that if unequal pressure was made in the preparation of dry films, some parts of the slide would show only oval cells and other parts mostly round cells; sometimes, in such a slide, groups of unusually small cells, circular in outline, were seen (fragmentation?). Our most interesting experiment of this sort consisted in putting pressure on the cover slip over a drop of blood (whole blood or oxalated or citrated blood) either before or after sealing with petrolatum. This procedure often resulted in a great reduction, sometimes in an almost complete disappearance, of oval forms, and their replacement by circular forms, some of them much smaller than normal, which did not regain their oval shape or normal size when the cover slip was gently tilted and replaced.

*Experiment 2.*—Was the oval shape of the erythrocytes present in the patient's circulation, or did it appear only after the blood left the vessels? The question could not be fully answered. As Ponder<sup>1</sup> said, no one knows the shape of human blood cells in the circulation, since there is no way of examining them within the vessels such as is afforded, for example, by the webbed foot of the frog. Our nearest approach to an answer was, perhaps, the following experiment: A drop of the patient's oxalated plasma, previously obtained, was placed on his finger, and a needle puncture was made through the drop; this was wiped off and a fresh drop of plasma applied into which a fresh drop of blood was gently squeezed. On immediate examination, practically all the cells in this preparation were oval.

*Experiment 3.*—Another experiment to the same end as No. 2 consisted in making a hanging drop of whole blood, sealing it and examining the erythrocytes that presently appeared in the expressed

serum as the clot formed; only oval cells appeared. Attention may be called here to the fact that at the autopsy slides made from the cardiac blood showed oval cells predominantly, and that microscopic sections showed oval cells in the splenic sinuses, capillaries of the cardiac muscle, suprarenal glands, etc.

We recall, also, that Huck and Bigelow's patient was used as a donor of blood for transfusion, and that the transfused cells kept their oval shape in the recipient's blood for a considerable time, as we infer from their statement that "no oval cells could be found after a period of two months."

*Experiment 4.*—Is the oval shape inherent and structural, or does it depend on some peculiarity of the patient's plasma? We did what others have done, placing washed cells from our patient in the serum of a normal person whose blood was of the same group, and this person's cells in our patient's serum. We found, as have others in like cases, that no change in the shape of the cells occurred in either preparation.

It seems clear that the peculiar shape depends on structure, a conclusion that scarcely requires experimental support, since in all reported cases both round and oval forms are found in the same environment, namely, the peripheral circulation. It must be remembered that no one has reported a case of 100 per cent elliptical erythrocytes. Why certain cells, sometimes only a few and sometimes almost all, are destined to take the oval shape, and where this shape is first assumed, are matters of which we remain ignorant. Puncture of the sternum by Bernhardt and by Cheney and our own examination of marrow from a femur have failed to show any site of origin of oval cells as such. Bernhardt's conclusion was that such cells appear first in the peripheral circulation. We, however, have been able to show that they are present in capillaries of the cardiac muscle, spleen and suprarenal glands.

In this connection, Bernhardt<sup>9</sup> made the following remark, which we consider valuable: "Clinically one must segregate this ovalocytosis from other clinical findings . . . and look upon it as an anomaly, a sort of atavism." This thought must have occurred to others who have encountered these extraordinary cases of oval erythrocytes in man; they seem to us as suggestive of atavism as the cases, also rare, of persistent branchial cleft.

At one time in the consideration of our first case, we thought that not only the oval shape of our patient's erythrocytes but also his blood group might be reminiscent of an ancient condition. Our patient's blood belonged to group A and the blood in all other cases in which the point was determined belonged to that group so far as we could determine at the time, a group that there is some reason for thinking was the

earliest mutation from group O, regarded by most writers as the original human group. But the blood of our second patient belonged to group O, and, as we now know from Hunter and Adams' cases and from Cheney's, all groups are represented. Nevertheless, we allow our far-fetched speculation to stand, thinking that these cases may yet, from one or the other approach, furnish a link between the marine vertebrates and man. The total number of known cases of elliptical erythrocytes in man is too small, even if the persons concerned and all their available relatives were investigated, to give us more than an intimation of a correlation between blood group and oval cell if such exists. The number of studies of three generations is only two, and these studies have not been complete, so far as we know, with respect to the blood groups.

Hetero-agglutination tests between man and other animals may in time throw some light on the subject. They have not yet been carried far, though, as Synder<sup>17</sup> said, a wide field is open here for the study of taxonomic relationships. Two recorded observations we here set down: 1. Landsteiner and Miller<sup>18</sup> reported that fourteen of seventeen chimpanzees examined had blood belonging to group A. 2. Karshner<sup>19</sup> found that human serum of group A seldom agglutinated the oval cells of the chicken—only eight times in 169 tests—while serum of group B never failed to agglutinate in 112 tests.

That even morphologic studies might lead to some clue or at least disclose some interesting and curious facts, if animal hematology were given the same intensive study that human hematology has received in the last fifteen years, is suggested by a paragraph that we find in an old journal wherein the author,<sup>20</sup> referring to the red cells of Mexican and Persian deer, spoke as follows: ". . . curved and gibbous in the middle and acutely pointed at the ends, with a concave and convex margin, like a crescent"—quite a number of such forms being present aside from the usual disks.

Does the occurrence of elliptical erythrocytes in man throw any light on the question, long debated, whether the human erythrocyte in its usual form is a cell the shape of which is maintained by a special structure, as Ponder<sup>21</sup> believes, or merely a fluid droplet the form of which is due to surface forces only, as maintained by Norris, Gough,<sup>22</sup> and

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17. Snyder, L. H.: *Blood Grouping*, Baltimore, Williams & Wilkins Company, 1929, p. 116.

18. Landsteiner, K., and Miller, C. P.: *J. Exper. Med.* **43**:860, 1925.

19. Karshner, W. M.: *J. Lab. & Clin. Med.* **14**:346, 1929.

20. Gulliver, George: *Abstr. Philos. Tr. Roy. Soc. London* **4**:199, 1840; *London & Edinburgh Philos. Mag.*, November, 1840, p. 329.

21. Ponder, Eric: *Quart. J. Exper. Physiol.* **14**:338, 1924.

22. Gough, Alfred: *Biochem. J.* **18**:202, 1923.

others? We think it suggests a probability of the correctness of Ponder's view. We have presented evidence to show that the elliptical human erythrocyte is such by structure, and that it is a functionally normal cell in normal, though exceptional, human beings—in other words that there are normal human erythrocytes the shape of which is due to structure.

If the usual, biconcave, disk-shaped erythrocyte is likewise such by structure, it would seem to be of simpler architecture than the biconcave oval and at the same time more efficient and more economical, as has been shown mathematically, for its principal function of oxygen transport and exchange. Here, again, one may guess that the elliptical human erythrocyte is an atavistic form, structurally such from some early necessity (say a theoretically less constant plasma), while the usual disk in man and most of the higher vertebrates is a later development adapted to conditions (say a fully evolved, species-specific plasma) in which such a degree of stability or rigidity of form is no longer necessary or advantageous. One sees a somewhat similar adaptation of cells and serum, still in progress and not yet completed, in the blood of the new-born child—not in any changing structure in the erythrocyte, but in the shifting relative strength of receptors in cells and serum, in neither of which is development likely to be complete at birth, at which time there is usually a relatively strong receptor development in the serum and a weak receptor development in the cell—plainly a temporary defensive mechanism without which antibodies in the maternal circulation might be bound on the erythrocytes of the child; a mechanism that continues for some time beyond its apparent usefulness until, some months after birth, permanent relations are attained between cells and serum and the blood group is established. Whether this stability is attained first in the cells or in the serum is not known. The matter is discussed by Thomsen<sup>23</sup> in an article on the quantitative development of group-specific substances in the serum of the new-born infant.

*Experiment 5.*—Sealed wet preparations were made (1) from fresh blood and (2) from citrated or oxalated blood and kept for long periods both at room temperature and in the icebox. Neither a reduction in the number of the oval cells nor any change in their shape was seen in any of these preparations in twenty-four hours; later, beginning as a rule in seventy-two hours, many of the cells became circular in outline. To establish this observation, we kept a fresh, wet, sealed preparation on the microscope stage unmoved for ninety-six hours. The change to round form that we speak of occurred much later in preparations kept in the icebox, beginning on the thirteenth day.

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23. Thomsen, Oluf: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:199, 1931.



*Experiment 6.*—Blood from the finger or from the ear was collected in capillary tubes about 15 cm. long, and the ends of the tubes were sealed. In these tubes, the blood clotted in a long thread, which could be drawn out when the tube was opened. In the remaining serum, which could then be blown out, erythrocytes could always be found. Gram<sup>24</sup> recommended this method for the preservation of cells for examination over long periods. We sent such tubes as far away as the Pacific coast, and we were told that on the arrival of the tubes there, oval cells were found in the serum.

*Experiment 7.*—We confirmed Bernhardt's observation<sup>9</sup> that in beginning hemolysis in hypotonic salt solution some of the oval cells take on the round form. But we can add that if the process is watched under the microscope, many oval shadows will be seen—oval cells that undergo hemolysis without change in shape. Some, too, if the cells are in motion, may be seen to lose their biconcavity and become flimsy, bending double and flattening out again while keeping their oval outline.

*Experiment 8.*—A change of shape from oval to round can take place, without hemolysis, as we showed in our first experiment. It can also take place, without hemolysis, under another condition: When the blood of our patient was repeatedly washed with isotonic salt solution and the sediment finally suspended in salt solution, it was found that the oval cells had largely disappeared and been replaced by round forms. In this respect, we think the oval cells behaved like the usual normal discoid human erythrocytes, which, as Gough<sup>22</sup> observed, confirmed by Ponder,<sup>21</sup> change to spheres when immersed in salt solution. Kanellis<sup>25</sup> showed that experimentally the poikilocytes in pernicious anemia are not changed in shape by washing with hypotonic, hypertonic or isotonic salt solution. We made a somewhat similar experiment in a case of profound secondary anemia (C. B., gastric carcinoma) and saw no change in the poikilocytes. This adds somewhat to the evidence that the oval erythrocyte is not a poikilocyte due primarily or secondarily to any disease, but is a congenital anomaly not incompatible with health.

*Experiment 9.*—On two occasions, our patient was given oxygen by means of the apparatus for measuring basal metabolism; the inhalation of oxygen made no change in the shape of his red cells.

*Experiment 10.*—Under the conditions of experiment 10 which we repeated several times, some cells may have changed from oval to

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24. Gram, H. C., quoted by Holler, G., and Kudela, O.: *Folia haemat.* **35**:97, 1928.

25. Kanellis, E.: *Folia haemat.* **35**: 71, 1928.

round, but we doubt if this fully explains the findings that we are about to record:

1. The patient's blood was collected in citrated isotonic salt solution and mixed by inverting the tube a few times without shaking; then it was centrifugated at low speed for two minutes. Wet sealed preparations were then made both from the upper part of the tube and from the bottom. The former showed mostly round cells; the latter, almost nothing but ovals.

2. Blood was taken from a vein into an excess of citrated salt solution and gently mixed. After standing an hour, a large proportion of the cells in the upper part of the tube were round, while most of those at the bottom were oval. The supernatant layer was then decanted into a second tube, and the two tubes were left standing over night. Next day the sediment of cells in the first tube still showed almost none but oval cells; no hemolysis had occurred in either tube. The second tube was gently shaken to bring the cells into suspension again and then centrifugated briefly at low speed until a partial clearing of the upper layer was seen. In the preparations from the upper portion, we found that 90 per cent of the cells were round. The contents were then decanted down to the last half cubic centimeter, and this sediment was found to be composed almost wholly of oval cells.

We could hardly escape the conclusion that both oval and round cells were present in our patient's circulation, and that the oval cells were the heavier.

*Experiment 11.*—Fragility of the erythrocytes was normal in cases reported by Huck and Bigelow, Bernhardt and Cheney. Our two cases call for some comment.

In the case of John C., hemolysis began at 0.5 per cent and was complete at 0.22 per cent, which is to be compared with 0.42 per cent and 0.32 per cent for the normal control done at the same time. In the case of J. S., hemolysis began in the test and the control at the same point, viz., 0.46 per cent, but the end-points were far apart—0.24 per cent for the patient and 0.36 per cent for the control done at the same time. In the case of J. S., we effected a separation of the round and the oval cells in the manner described, and repeated the test on the two fractions, the suspensions being first standardized as to opacity. The result was that cells from the upper layer (mostly round) agreed with the control in the point of beginning hemolysis (0.46 per cent), but went a little farther to the end-point, which was 0.32 per cent; whereas cells from the bottom of the tube (mostly oval) did not begin to hemolyze short of 0.42 per cent and were not completely hemolyzed short of 0.24 per cent, the end-point agreeing, as may be seen, with that of the patient's whole blood. When the like experiment was done

with normal blood, the fragility of the cells from the upper layer was identical with that of the cells from the bottom of the tube.

In both of our patients, therefore, some of the erythrocytes appeared to be less resistant and some more resistant than the whole blood of the controls, and in the case of J. S. we were able to show that the cells that were the more resistant were the oval cells.

#### SUMMARY

Fifty-two instances of elliptical human erythrocytes are collected, including two of our own. An analysis of the cases establishes the condition as a hereditary anomaly not incompatible with health and without any proved relation to disease, occurring in whites, blacks and mulattos, and about equally divided between males and females. Both sexes may transmit it. A generation may be skipped. All four blood groups are represented.

An autopsy is reported showing that oval cells were present in the smallest vessels of the cardiac muscle, spleen and suprarenal glands. An occasional oval erythrocyte was found in the marrow of the femur, and the marrow there, instead of having the usual yellow color and thick consistency, was reddish purple and semifluid or lymphoid. In other respects, the autopsy disclosed nothing more than was anticipated from the patient's clinical condition, which was chiefly that of severe nephritis.

Certain experiments are presented and discussed. Some of them were merely such as were necessary to establish the validity of the cases reported. Others yielded the following observations: 1. When the blood of one of the patients was subjected to the action of hypotonic salt solution, the oval cells resembled in their behavior normal erythrocytes rather than the poikilocytes of a patient with anemia. 2. Both round and oval cells were shown to exist together in the peripheral circulation, and the oval shape was shown to depend on structure and not on environment. 3. It was found possible to separate the two kinds of cells and to do certain experiments that showed that the oval cells were heavier than the round cells, and that they were more resistant than the round cells to the hemolytic action of hypotonic salt solution.

A speculation is offered as to the possible atavistic significance of the occurrence of elliptical erythrocytes in man.

# LESIONS OF THE NERVOUS SYSTEM RESULT- ING FROM DEFICIENCY OF THE VITAMIN B COMPLEX\*

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It has been known for a long time that deficiency of vitamin B<sup>1</sup> leads to well defined neuromuscular symptoms. In animals, these are manifested as spastic paralysis, ataxia, opisthotonus and convulsions. In 1897, Eijkman<sup>2</sup> described noninflammatory atrophic degeneration of the medullary sheaths of the nerves in hens fed on a diet of polished rice. He also noted chromatolysis and atrophy of the ganglion cells of the anterior horns of the spinal cord. Vedder and Clark<sup>3</sup> in 1912 made similar observations in rice-fed fowls. They found, in addition, chromatolysis in the dorsal and ventral root ganglions and degeneration in the nerve roots and spinal cord involving the myelin sheaths and axis cylinders. Pigeons fed on autoclaved rice were found by McCarrison<sup>4</sup> to have degenerated fibers throughout the spinal cord and in the nerve roots. Furthermore, the sciatic nerves in his animals showed degenerated fibers in 88 per cent of the cases, and the vagus nerves in about 63 per cent. He concluded that the paralytic symptoms were due mainly to impaired functional activity of nerve cells and much more rarely to their degeneration. Findlay,<sup>5</sup> in an experimental study on avian beriberi, found a complete disappearance of the Nissl

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1. In this paper, the term vitamin B refers to the mixture of water-soluble vitamins composed of the heat-labile antineuritic factor (vitamin B<sub>1</sub>) and the heat-stable pellagra-preventive factor (vitamin B<sub>2</sub>). The terms vitamin B<sub>2</sub> and vitamin G are used synonymously.

2. Eijkman, C.: Eine Beri Beri-Ähnliche Krankheit der Hühner, *Virchows Arch. f. path. Anat.* **148**:523, 1897.

3. Vedder, E. B., and Clark, E.: A Study of Polyneuritis Gallinarum: A Fifth Contribution to the Etiology of Beriberi, *Philippine J. Sc.* **7B**:423, 1912.

4. McCarrison, R.: The Pathogenesis of Deficiency Disease, *Indian J. M. Research* **6**: 275, 1919.

5. Findlay, G. M.: An Experimental Study of Avian Beriberi, *J. Path. & Bact.* **24**:175, 1921.

bodies in the nerve cells of the spinal cord and evidence of nuclear degeneration and myelin disintegration in the sciatic and vagus nerves. He stated that in two pigeons that died of chronic inanition demyelination was present in the sciatic nerves.

The first work of importance conducted on mammals was that of Voegtlin and Lake,<sup>6</sup> who produced polyneuritis in cats by feeding alkali-treated, autoclaved meat. The changes that they observed consisted of myelin degeneration of the sciatic nerves similar to polyneuritis gallinarum, and of degenerated fibers at all levels of the spinal cord.

It is difficult to attribute the changes described in the early work solely to lack of the antineuritic vitamin, owing to the fact that the rations used in these experiments were deficient in more than one essential nutrient, namely, protein (rice diets), minerals and other vitamins. The animals were therefore suffering from multiple dietary deficiencies.

Later investigations on this question have limited the regimen more definitely to lack of vitamin B alone. It is a well known fact that the absence of this vitamin leads to a loss of appetite and consequently to voluntary starvation (Cowgill<sup>7</sup>). This factor was taken into consideration by Woollard,<sup>8</sup> who adequately controlled the effects of inanition. In rats fed an artificial diet complete so far as known except with respect to undifferentiated vitamin B, he found changes in the intermuscular medullated motor and sensory nerves and their endings. In the control animals, totally deprived of food but receiving a sufficient amount of autolyzed yeast, similar but less extensive changes were observed.

In recent years it has been found that what has hitherto been called vitamin B is really a mixture of at least two components, namely, the heat-labile antineuritic factor and a thermostable substance, the absence of which is thought to produce pellagra (Goldberger and others,<sup>9</sup> Chick and Roscoe<sup>10</sup>). Stern and Findlay<sup>11</sup> studied the changes in the nervous

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6. Voegtlin, C., and Lake, G. C.: Experimental Mammalian Polyneuritis Produced by a Deficient Diet, *Am. J. Physiol.* **47**: 558, 1919.

7. Cowgill, G. R.: A Contribution to the Study of the Relation Between Vitamin-B and the Nutrition of the Dog, *Am. J. Physiol.* **57**:420, 1921.

8. Woollard, H. H.: The Nature of the Structural Changes in Nerve Endings in Starvation and in Beriberi, *J. Anat.* **61**:283, 1927.

9. Goldberger, J.; Wheeler, G. A.; Lillie, R. D., and Rogers, L. M.: Experimental Black Tongue and the Black Tongue Preventive Action in Yeast, *Pub. Health Rep.* **43**:657, 1928.

10. Chick, H., and Roscoe, M. H.: The Dual Nature of Water-Soluble Vitamin B, *Biochem. J.* **22**:790, 1928.

11. Stern, R. O., and Findlay, M.: The Nervous System in Rats Fed on Diets Deficient in Vitamins B<sub>1</sub> and B<sub>2</sub>, *J. Path. & Bact.* **32**:63, 1929.

system of rats fed diets deficient in vitamin B<sub>1</sub> (antineuritic component) and B<sub>2</sub> (pellagra-preventive factor), respectively. In the first group of animals, they found chromatolytic changes in the ganglion cells of the spinal cord and early degeneration of the myelin of the peripheral nerves. In the second group, the changes consisted of swelling and vacuolization of the anterior horn cells of the spinal cord with the deposition in them of lipochrome pigment.

Gildea, Kattwinkel and Castle<sup>12</sup> attempted to correlate the clinical manifestations of deficiency of the B factors with anatomic alterations of the nervous system commonly noted in pernicious anemia. For this purpose, they used dogs that were fed the Cowgill diet<sup>13</sup> and described lesions in the cords of these animals that resembled those seen in so-called combined system disease.

The majority of the investigators in this field heretofore have utilized the pigeon or the rat as the experimental animal. No complete investigation of the nervous system, peripheral as well as central, has been conducted on the dog. In view of the extensive work done on this animal by Cowgill and by Goldberger in their studies on the relation of the water-soluble B vitamins to nutritional disease, it appeared to be of interest to see whether the anatomic basis for the clinical manifestations observed in the dog was similar to that already described in the other species. Because it had been claimed by Marrian, Baker, Drummond and Woollard<sup>14</sup> that the disorders found in B-avitaminosis are due entirely to the accompanying factor of inanition, and because these investigators had described a peripheral polyneuritis in rats deprived of food completely, it was important to study further the rôle of inanition in producing these anatomic lesions.

#### EXPERIMENTAL PROCEDURE

*Diets Employed.*—Five of the twelve animals used in this study were allowed to subsist from the beginning of the experiment on the artificial diet described by Cowgill,<sup>13</sup> supplemented, however, with 0.3 cc. of cod liver oil per kilogram per day. The composition of the ration is shown in table 1.

It should be pointed out that although this diet is deficient in the vitamin B complex, the changes observed in the study here reported are related essentially to lack of the antineuritic component. The ration is low in its content of the heat-stable B<sub>2</sub> substance, but not altogether devoid of it. The commercial casein

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12. Gildea, E. F.; Kattwinkel, E. E., and Castle, W. B.: Experimental Combined System Disease, *New England J. Med.* **202**:523, 1930.

13. Cowgill, G. R.: Studies in the Physiology of Vitamins, *Am. J. Physiol.* **66**:164, 1923.

14. Marrian, G. F.; Baker, L. C.; Drummond, J. C., and Woollard, H. H.: Physiological Rôle of Vitamin B: Relation of Inanition to Vitamin B Deficiency in Pigeons, *Biochem. J.* **21**:1336, 1927.

employed was shown by Evans and Burr<sup>15</sup> to contain an appreciable amount of the thermostable vitamin (B<sub>2</sub>). How effective small amounts of the missing essential, when taken daily in the diet, may be in delaying symptoms of a deficiency disease is demonstrated by the experiments of Goldberger and his collaborators,<sup>16</sup> in which they observed that increase in the amount of acid-leached casein in the diet suspended considerably the onset of the symptoms of B<sub>2</sub>-deficiency in dogs. As the casein used in the diet here described was not further purified by acidulated water extraction, as was done in the experiments of Goldberger and his associates, the ration must have carried a significantly greater amount of the B<sub>2</sub> factor. Furthermore, the time required for the development of pellagra-like manifestations is usually longer than that found necessary for the production of polyneuritis. These studies were carried to the point at which neuritic symptoms occurred, at which

TABLE 1.—*Casein III Diet*

	Per Cent	Calories
Casein, commercial (81.9 per cent protein).....	41.2	136
Sucrose.....	29.4	118
Lard.....	18.3	165
Butter *.....	7.2	58
Salt mixture †.....	1.3	...
Bone ash.....	2.6	...
Total #.....	100.0	477

\* Estimated as containing 90 per cent fat.

† Karr-Cowgill<sup>17</sup> salt mixture.

# One gram of this diet is therefore equivalent to 4.8 calories.

TABLE 2.—*Purified Casein Diet \**

	Per Cent	Calories
Casein† (87.1 per cent protein).....	29.0	101
Sucrose.....	33.0	132
Lard.....	27.5 }	302
Butter fat.....	0.1 }	
Salt mixture ‡.....	1.6	
Bone ash.....	2.8	
Total §.....	100.0	535

\* This ration was supplemented with 0.3 cc. of cod liver oil per kilogram per day, and with sources of antineuritic vitamin B.

† In this instance, casein refers to a highly purified product devoid of the water-soluble vitamin factors, obtained from the Harris Laboratories, Tuckahoe, N. Y.

‡ Karr-Cowgill<sup>17</sup> salt mixture.

§ One gram of this diet is therefore equivalent to 5.4 calories.

time no signs of vitamin B<sub>2</sub> deficiency, such as bloody diarrhea, cutaneous sores, buccal lesions and intense salivation (Goldberger and others<sup>9</sup>), were manifested.

Three of the eight animals (nos. 1, 5 and 6) on the deficient rations had a different nutritive history. These dogs had been used for another experiment, in which the ration employed was highly purified with respect to the heat-stable B<sub>2</sub> substance (table 2).

15. Evans, H. M., and Burr, G. O.: A New Differentiation Between the Antineuritic Vitamin B and the Purely Growth-Promoting Vitamin B, *J. Biol. Chem.* **77**:231, 1928.

16. Goldberger, J.; Wheeler, G. A.; Rogers, L. M., and Sebrell, W. H.: A Study of the Black Tongue Preventive Value of Leached Commercial Casein, Together with a Test of Black Tongue Preventive Action of a High Protein Diet, *Pub. Health Rep.* **45**:273, 1930.

The opportunity was presented to transfer two of these animals (nos. 5 and 6) to the casein III regimen, with a view to studying changes in the nervous system as affected by previous depletion of vitamin B<sub>2</sub>. Dog 1 subsisted entirely on the highly purified casein diet. When symptoms of antineuritic vitamin B deficiency were manifested in these animals, a bloody diarrhea was observed in every case, indicative of a lack of the heat-stable factor.

*Details of Feeding.*—The dogs were given an allotment of food once a day in sufficient amount to maintain the body weight. Their intakes of food were determined daily, in order to see whether the severity of the pathologic process, as exemplified by damage to nerve tissue, could be correlated with the degree of anorexia characteristically exhibited by vitamin-B deficient animals.

*Fasting Controls.*—In addition, two animals (nos. 11 and 12) were entirely deprived of food, but given 1 Gm. of vitavose per kilogram per day as an adequate, exogenous source of the vitamin B complex. It was previously determined by Cowgill<sup>17</sup> that a daily intake of 0.6 Gm. of vitavose is sufficient to maintain perfect appetite in animals subsisting on the casein III diet. This dosage was raised to 1 Gm. in order to insure a reasonable factor of safety.

*Normal Controls.*—Two animals (nos. 9 and 10) were on diets complete in all respects and at no time displayed any manifestations of nervous disease.

*Postmortem Examinations.*—It was felt that destructive changes could be more readily detected if the pathologic process was made as severe as possible. The animals were therefore killed as near the point of death as could be judged, or were allowed to proceed to a fatal termination. Complete postmortem examinations were performed on the animals within four hours in every case. These included an examination of the sciatic nerves, the brachial plexuses, the median and ulnar nerves, the vagi and the whole brain and spinal cord with the nerve roots. In each instance, the paired nerves were examined for comparative purposes.

*Histologic Technique.*—Blocks of all organs (except the nervous system) found abnormal in the gross were fixed in Zenker's fluid to which acetic acid had been added and in a diluted solution of formaldehyde, U. S. P. (1:10), for staining with hematoxylin-eosin. The nervous system in each instance, both central and peripheral, was sectioned on removal from the body and fixed in 95 per cent alcohol, in a diluted solution of formaldehyde, U. S. P. (1:10), and in Müller's solution. The alcohol-fixed material was embedded in celloidin and stained by the original Nissl method (toluidine blue), with hematoxylin-eosin and by the Klarfeld tannic acid-silver carbonate method, when indicated. The formaldehyde-fixed material was employed in part for sudan III stains for fat, for the demonstration of axis cylinders by the Bielschowsky method and for the demonstration of myelin sheaths by the Spielmeyer method, and in part for the study of myelin sheaths by the Kulschitsky modification of the Weigert method. The material fixed in Müller's solution was stained with osmic acid, embedded rapidly in celloidin, and sectioned at 30 microns. In this way, it was hoped to obtain both a positive and a negative picture of any possible myelin degeneration.

#### EXPERIMENTAL RESULTS: CLINICAL FINDINGS

The length of the experimental period varied with the individual animal, but in dogs subsisting on casein III the symptoms usually

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17. Cowgill, G. R.: An Improved Procedure for Metabolism Experiments, J. Biol. Chem. **56**:725, 1923.



appeared within from sixty to ninety days after the vitamin B complex was withheld.

The pathologic manifestations of vitamin B-deficiency as they occur in the dog have been described by Cowgill.<sup>7</sup> The first symptom is a dragging of the hind legs when the animal lifts itself upon its feet. These limbs remain extended when the dog walks, so that the typical reflex of extensor-flexor alternation is not exhibited. This maintained contracture of the hind limbs becomes more and more marked; the toes are curled in, so that the animal stands on its knuckles. Subsequently, a pronounced ataxia is manifested when the dog is allowed to walk.

The spastic paralysis progresses cephalad; eventually the fore limbs become involved, and finally the neck muscles. A marked opisthotonos is exhibited. The entire nervous system becomes hypersensitive, and tonic spasms are frequent.

In the advanced stages of the disease, severe, generalized convulsions are present. In some of the animals, the convulsions occur fairly soon

TABLE 3.—*Survival Period of Dogs on Vitamin B-Deficient Diets*

Dog	Survival Period, Days
1.....	1
2.....	7
3.....	5
4.....	1
5.....	3
6.....	2
7.....	1
8.....	3

and are the dominant part of the syndrome. These clonic spasms, which recur intermittently between periods of relaxation, are not unlike the picture produced by strychnine poisoning.

The period of survival after the appearance of the symptoms varied in this study between one and seven days, as table 3 indicates.

In general, it may be said that the survival period was shorter for those animals that manifested the convulsive seizures early. On the other hand, when the condition of progressive tonic spasticity was the first feature, the number of days the animals lived after the first appearance of the symptoms depended on how soon the clonic spasms occurred.

The syndrome exhibited by dog 4 was somewhat atypical. On the forty-fourth day, a convulsion occurred, involving the facial muscles. Frothing at the mouth and the nature of the convulsion suggested an epileptiform attack. On the fifty-first day, two such seizures occurred, and throughout the following morning they were exhibited in rapid succession between very short periods of relaxation, and resulted in the death of the animal early in the afternoon.

Dogs 1, 5 and 6 had been previously depleted of the heat-stable B<sub>2</sub> substance (see Experimental Procedure). They all vomited on the day preceding the neuritic manifestations. The foul breath and bloody diarrhea, which were accompanying features, suggested an alimentary disturbance, probably due to lack of vitamin B<sub>2</sub>.

#### ANATOMIC FINDINGS

Findings other than those in the nervous system were but incidental to the purposes of this study and are recorded only in the individual protocols of these animals. On the basis of the histologic changes in the central nervous system, the eight dogs maintained on the vitamin



Fig. 1.—Drawing of a microscopic preparation of sciatic nerve of dog 6; Marchi;  $\times 220$ .

B-deficient diets can be divided into two groups: group 1, comprised of dogs 1, 5 and 6, which were previously on a vitamin B<sub>2</sub>-deficient diet, and group 2, comprised of dogs 2, 3, 4, 7 and 8, the ration of which lacked essentially the antineuritic factor.

*Group 1.*—On gross examination of dogs 1, 5 and 6, no abnormalities were noted in the covering of the brains and spinal cords. Multiple frontal sections of the cerebral hemispheres revealed no deviations from the normal in color and consistency. The brain stems and cerebella were similarly without change. Numerous transverse sections of the spinal cords at all levels revealed no anemia, hyperemia or softening. Likewise, all the peripheral nerves, including the vagi, were quite like those of normal animals.

In the Marchi preparations of the peripheral nerves, numerous clumps of black granules could be seen in most of their fibers (fig. 1). Often these dark bodies were concentrated at points where the fibers appeared to be swollen, and they lay within, and not outside of, the neurilemmas. In the same fiber they often appeared at several points, with granule-free zones intervening. The distal as well as the proximal portions of the nerves were equally involved, as were even portions of the brachial plexuses. In all three animals, the vagi showed the same myelin degeneration but to a less degree, and in general the median



Fig. 2.—Photomicrograph of sciatic nerve of dog 6, showing extensive demyelination. A few fibers only show no degeneration along their whole course; Spielmeier;  $\times 60$ .

and ulnar nerves were somewhat less involved than the sciatic nerves. The most extensive changes of this character were present in dog 1, in which, also, the sciatic nerves showed more involvement than the other nerves examined.

Spielmeier and Kulschitsky preparations of the same nerves showed comparable pictures of extensive demyelination (fig. 2). The widespread involvement noted in the Marchi preparations was confirmed by these stains. Again it could be noted that not invariably was the whole nerve fiber destroyed, but that along the course of a single fiber degen-

erated portions alternated with nearly normal portions. Some of the least involved fibers had a foamy appearance, but stained nearly as well as the completely normal fibers. Others showed swelling at irregular intervals, stained capriciously, and often contained round, gray granules of partially disintegrated myelin. Many of the individual nerve fibers failed to stain at all by either of these two methods.

In preparations stained with sudan III (fig. 3), the myelin sheath degeneration was again confirmed. In dogs 5 and 6, the peripheral nerves thus stained were seen to contain large and small clumps of granules of a brilliant orange-red color. Little or no fat was found lying between the nerve fibers; all seemed to be encased within the sheaths of Schwann. None of the fat was phagocytosed; indeed, no



Fig. 3.—Drawing of sciatic nerve of dog 6; sudan III;  $\times 220$ .

phagocytic cells were present in the nerves of these two animals. In dog 1, on the other hand, most of the brightly stained fat particles from the degenerated myelin sheaths in the sciatic nerves were phagocytosed in fat-granule cells, which were present in abundance (fig. 4). The vagi of dog 1 lacked fat-granule cells, although demyelination was fairly widespread and demonstrable with sudan III. Thus there is ample proof of an extensive degeneration of myelin in the vagus, sciatic, median and ulnar nerves and in the brachial plexuses of dogs 1, 5 and 6. This demyelination was present in the paired nerves to an approximately equal degree, but was most marked in the sciatics and least marked in the vagi. Moreover, in all the stains employed, the degenerative process displayed a striking parallelism as regards severity of involvement. It was impossible to determine whether the sensory

or motor components of the peripheral nerves were predominantly involved, but that both were involved was more than likely, as some nerves showed degeneration of nearly all the individual fibers.

There is no question but that the neurilemmas of the destroyed nerve fibers appeared more cellular, but there is some doubt that this was due to an actual proliferation of the cells of the sheaths of Schwann. It is conceivable that the atrophy of the nerve fibers following the disappearance of the myelin sheaths could produce a condensation of the Schwann cells to simulate an increase in their number. No cells undergoing mitotic division were demonstrable. Also, with an atrophy of many of the nerve fibers, the intervening connective tissue cells were brought into prominence. That many of these supposedly neurilemmal

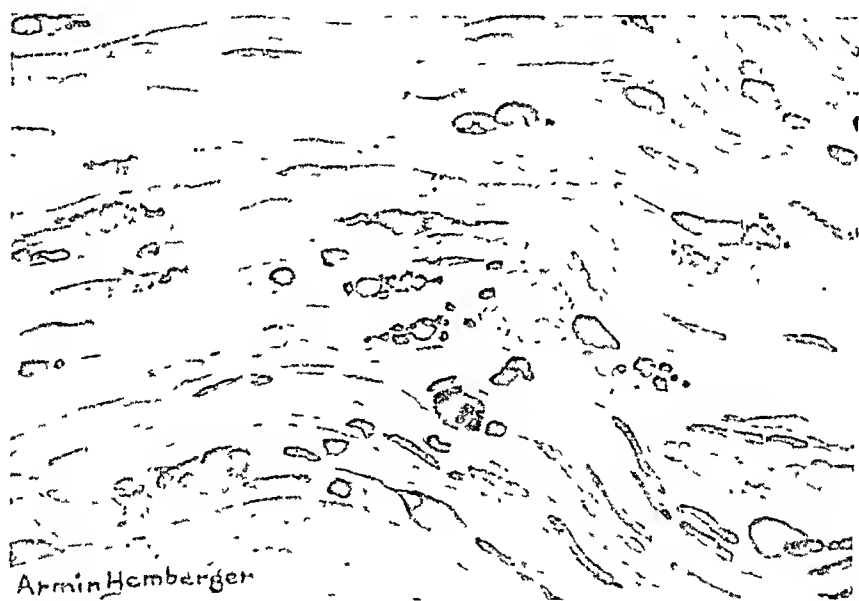


Fig. 4.—Drawing of sciatic nerve of Dog 1, showing fat from degenerated medullary sheaths phagocytosed by fat-granule cells; sudan III;  $\times 220$ .

cells had an elongated, oval shape like fibroblasts may not be without reason.

In contrast to the widespread and extensive demyelination of the peripheral nerves, the axis cylinders were intact almost without exception. That this was true for those nerves the myelin sheaths of which deviated but so slightly from the normal as to have a foamy appearance in the Spielmeyer stain or an occasional granule of fat in the sudan III stain is perhaps not surprising. But that the axis cylinders should be intact even in nerve fibers that had lost nearly all their myelin sheaths is a noteworthy finding. Only an occasional axis cylinder was fragmented or degenerated, and then only in the most severely demyelinated nerve fibers.

The spinal nerve roots, dorsal as well as ventral, showed no evidence of demyelination. The ganglion cells of the ventral and dorsal horns of the spinal cord in the Nissl preparations revealed well stained Nissl bodies. The nuclei had prominent, well stained nucleoli, and lay in central positions within the cells. There was no pigmentation of the cellular cytoplasm and no chromatolysis. The whole length of Goll's column in dog 1 was replaced by a marked glial reaction in which were present fibrillary astrocytes, rod-shaped Hottenga cells and myeloclasts, the latter in predominant numbers (fig. 5). Fat-granule cells were



Fig 5—Photomicrograph of dorsal columns in the spinal cord of dog 1. Note the triangle-shaped glial reaction in the median dorsal fasciculi, Nissl,  $\times 45$ .

absent. This glial response had a wedge-shaped outline with the base toward the pial surface, and lay in a symmetrical position on each side of the posterior median septum. In neither of the other two dogs was any such reaction present in the white matter of the spinal cord.

The Kulschitsky preparations were entirely negative for evidences of degeneration in dogs 5 and 6, and in areas other than the fasciculus gracilis in dog 1. In this fasciculus, the myelin destruction was unquestionable, and corresponded identically with the shape and position of the glial reaction noted in the Nissl stains (fig 6). It was sur-

prising, therefore, in view of the otherwise negative findings in the Kulschitsky stains, to find in the Spielmeyer preparations of all three animals that there were numerous large, irregular, patchy, unstained areas that superficially resembled demyelination (fig. 7). Their patchy distribution was that of a combined system disease, but the myelin sheaths in them did not have the appearance of destruction. Rather, these sheaths seemed simply to have failed to stain, but were visible as apparently intact structures under high magnification. This point was settled in the Marchi preparations of the spinal cord, where no degen-

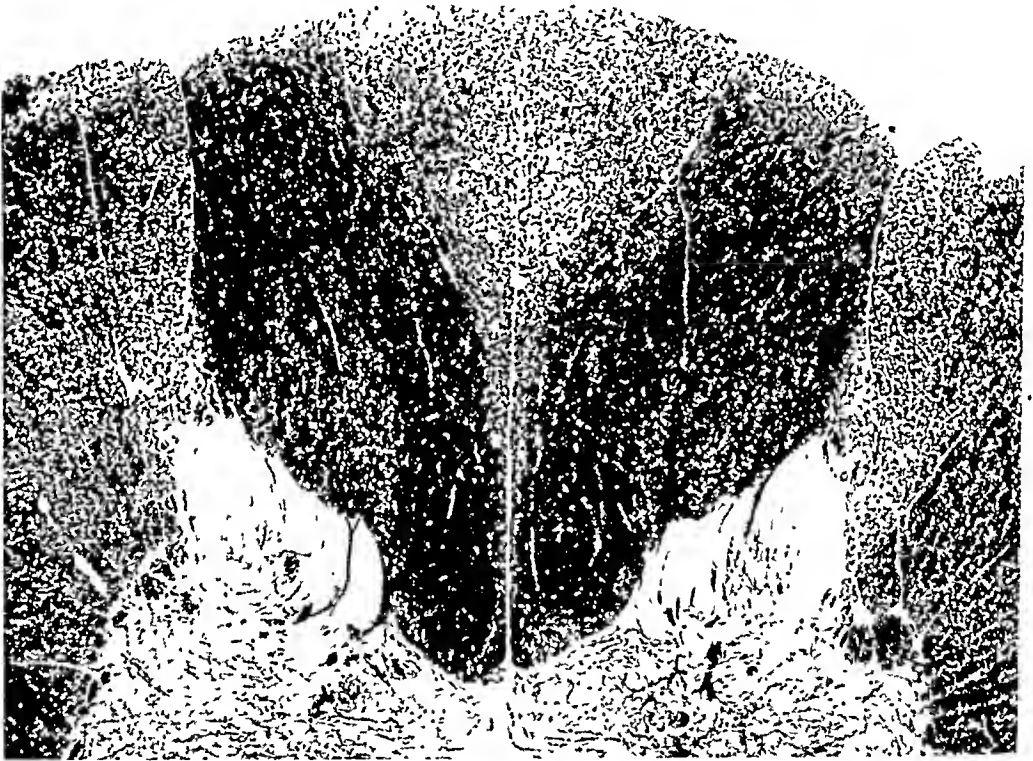


Fig. 6.—Photomicrograph of spinal cord of dog 1, showing demyelination in the median dorsal fasciculi; Kulschitsky;  $\times 40$ .

erated sheaths were found except in the medial dorsal columns of dog 1. To be sure, an isolated black granule was found here and there in other parts of the white matter, but it invariably lay outside of a sheath and was of an extraneous source. Similarly devoid of evidence of myelin destruction in the spinal cords were the sudan III stains, which failed to demonstrate fat even within the medial dorsal columns. Another point that needs mention is the fact that preparations in serial section of blocks of spinal cord stained by the Spielmeyer method never showed the unstained areas in precisely the same place in two consecutive preparations. These facts prove that there was no genuine com-

bined system disease in these animals, as the Spielmeyer preparations would appear to indicate.

The cortical cyto-architecture of the cerebral hemispheres as seen by the toluidine blue stain was completely normal. The vast majority of cortical nerve cells were stained well, had centrally placed nuclei, and contained the usual Nissl apparatus. Only a rare ganglion cell showed acute swelling with vacuoles in its cytoplasm. A particularly normal picture was presented by the large cells of Betz. No abnormalities were found in any of the nerve cells of the basal ganglions. The first



Fig. 7.—Photomicrograph of spinal cord of dog 6, stained by the Spielmeyer method. Note numerous patchy unstained zones (cf. fig. 13) similar to those observed in "combined system disease";  $\times 16$ .

deviation from the usual picture was encountered in all three animals in the region of the substantia gelatinosa rolandi (fig. 8). This bilateral lesion consisted of an extensive destruction of these zones with a marked vascular as well as glial proliferation. Many vessels of capillary size had large, swollen endothelial cells, the nuclei of some of which were in some stage of mitotic division. That these small vessels were greatly increased in number was excellently demonstrated in the Klarfeld preparations. The glial cells were of several types, with various forms of Hortega cells and oligodendroglia predominating. Many of



the transformed microglia were present as large, round mononuclear cells containing phagocytosed débris in their cytoplasm. Immensely swollen oligodendroglia cells were found in large numbers; these were often several times greater in size than the phagocytic microglia. They had small, eccentrically placed nuclei and an abundance of cytoplasm, which had a diaphanous, foamy appearance (fig. 9).

The whole cerebellum was devoid of changes, except for the vermis. This structure in all three animals was involved in an equal manner and to an equal degree. The mode of involvement was identical with

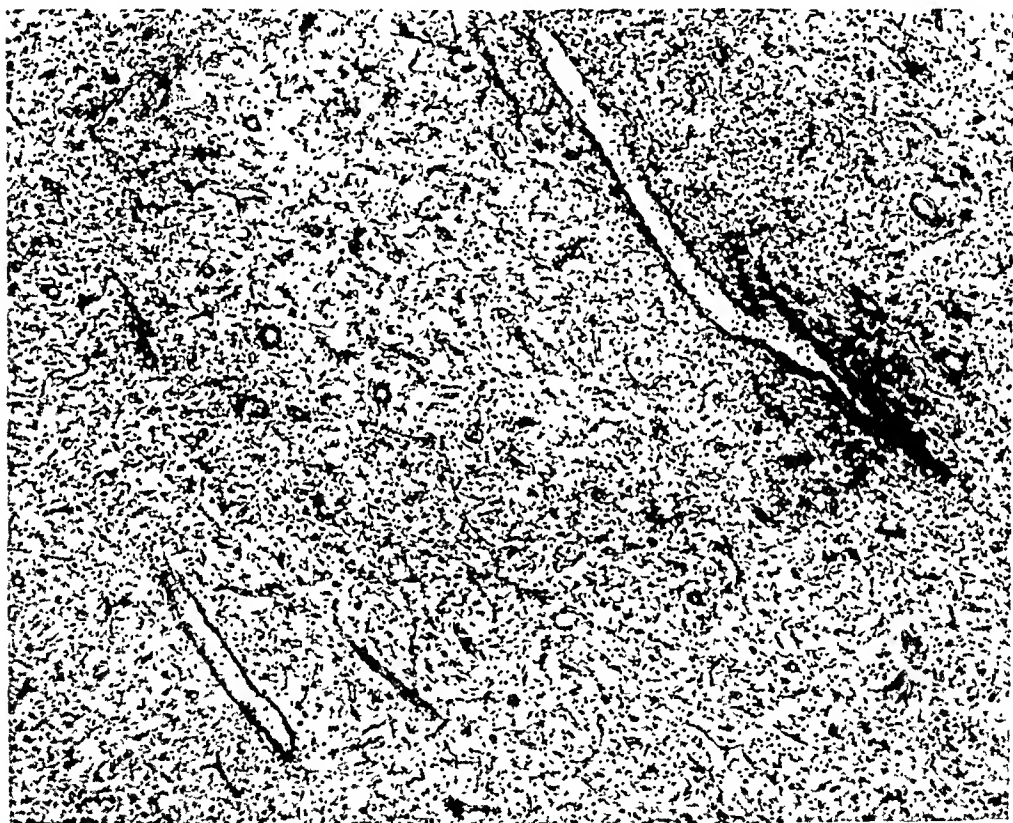


Fig. 8 (dog 5).—Photomicrograph of cellular and myelin destruction and glial and vascular proliferation in region of substantia gelatinosa rolandi; Nissl;  $\times 60$ .

that in the substantia gelatinosa—there were regressive glial and vascular proliferation (fig. 10). In the implicated part, the Purkinje cells were completely destroyed, and the granular cells were decreased in number, presenting a moth-eaten appearance. Except for the vermis and for the regions of the substantia gelatinosa of the pons and medulla, all the fat and myelin sheath stains of the cerebra, ponti, cerebella and medullae were negative.

*Group 2.*—As was the case in the animals of group 1, no changes were found grossly in the nervous systems of the animals in group 2.

Microscopically, however, lesions were also encountered in these animals. The peripheral nerves showed myelin destruction that compared closely with that found in group 1. In addition, however, the interesting fact came to light that there was a definite parallelism between the length of time the clinical symptoms of paralysis persisted and the extent of the anatomic lesion in the nerves. Dog 2 (symptoms for seven days), for example, showed more marked destruction of the sciatic nerves than any other animal in the series. Its median, ulnar and vagus nerves were more extensively destroyed than were the same

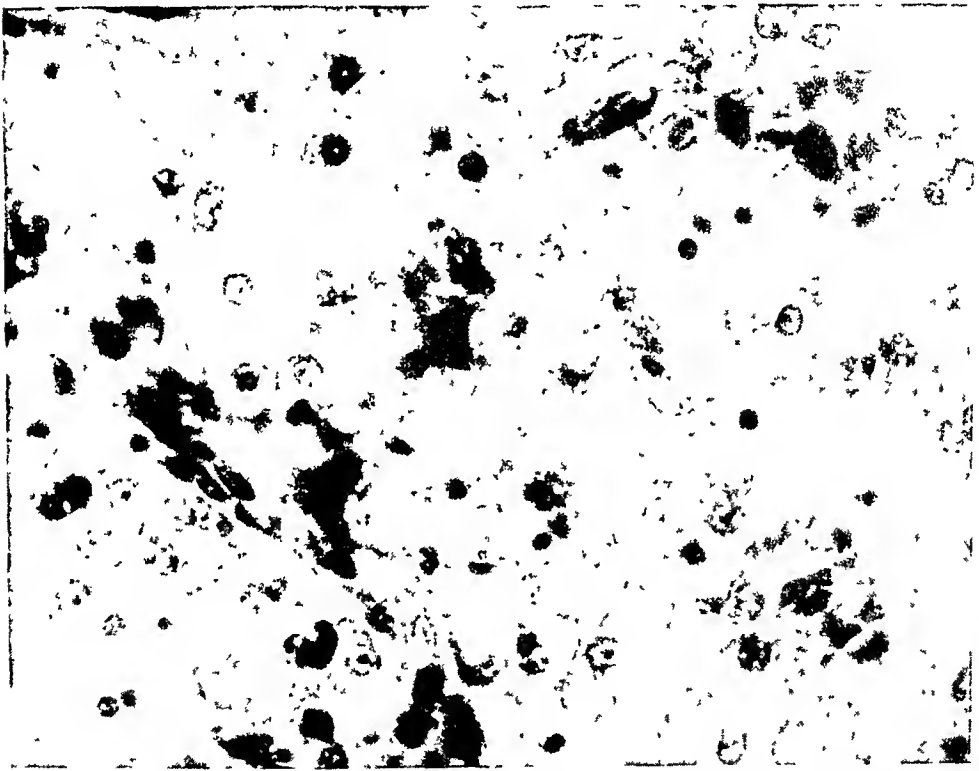


Fig. 9 (dog 5).—Photomicrograph of fat-granule cells and swollen oligodendroglia in region of substantia gelatinosa rolandi; Nissl;  $\times 550$ .

nerves in other animals. Again, the demyelination in dog 3 (symptoms for five days) was more extensive than in animals 4 and 7, which had symptoms for but a single day. That the parallelism between the severity of the anatomic lesion and the duration of symptoms was not entirely complete was demonstrated by dog 1 (symptoms for one day), the peripheral nerve lesions of which almost approached in severity those of dog 2. However, dog 1 had subsisted throughout the entire experimental period on the more highly purified casein ration (table 2), and for this reason it is unfair to compare the lesions of this animal with those in group 2.

There was no uniform change in the ganglion cells of the ventral and dorsal horns of the spinal cords. Indeed, the majority of these cells presented the modal picture. An occasional cell, in the midst of a group of normal ones, was unusually deeply stained, and its Nissl bodies were obscured. Very few cells showed acute swelling. The impression was gained that in spite of these minor changes in the ganglion cells no definite deviation from the normal was present in the anterior and posterior horns. In dog 4, an occasional ganglion cell



Fig. 10 (dog 5).—Photomicrograph of the degenerative process in the vermis cerebelli, showing loss of Purkinje cells and marked alteration of the granular cell layer. Note the increase in the number of blood vessels; Nissl;  $\times 45$ .

in the anterior motor horn was deeply stained, its cellular contents were hardly recognizable, and it had a few pericellular incrustations.

The Marchi, Kulschitsky and sudan III stains were all negative for myelin destruction in the spinal nerve roots as well as in the spinal cords themselves. The Spielmeyer method alone yielded results of the type described for group 1 and illustrated in figure 7. All these stains when applied to the cerebrum, cerebellum and brain stem yielded negative results.

The cerebral cortical cellular architecture was intact in all the animals of this group, except in dog 4. In this animal, which had epileptiform convulsions, numerous zones were found in the cerebral cortex where there was a partial or complete loss of the cortical laminae. One such area is shown in figure 11, where the paleness of the gyrus in the paracentral lobule was produced by a diffuse destruction of ganglion



Fig. 11 (dog 4).—Photomicrograph of cerebral cortex showing diffuse loss of ganglion cells in the crest of a gyrus; Nissl;  $\times 18$ .

cells of an ischemic type. These ischemic changes, unaccompanied by any demonstrable organic vascular lesions, were widespread. The cornu ammonis formation was no more extensively involved than were some lobules in the frontal, parietal and occipital lobes.

*Fasting Controls.*—In both animals of the fasting group, no changes were found on gross examination of the nervous system. Microscopi-

cally, however, the peripheral nerves showed a moderate degree of demyelination in the myelin sheath stains. In confirmation of this, the Marchi method applied to the nerves revealed a considerable number of black granules in dog 12 (fig. 12) and a small number in dog 11. But in the sudan III preparations only a very small amount of stainable fat was demonstrable.

The spinal nerve roots failed to show myelin destruction by any of the methods employed. Cross-sections of the spinal cords, however, revealed an occasional black ringlet by the Marchi method, representing

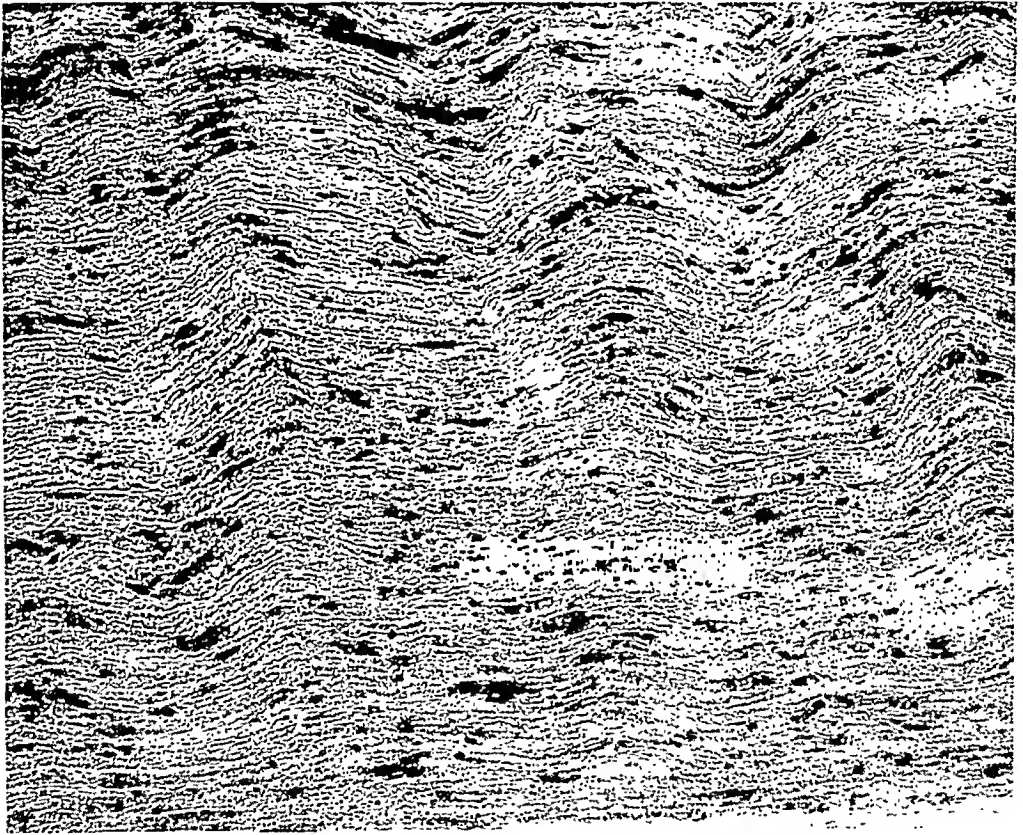


Fig. 12.—Photomicrograph of sciatic nerve of dog 12, showing numerous black granules of myelin degeneration by the Marchi method;  $\times 125$ .

a degenerated sheath. There was no uniformity nor apparent seat of predilection to the occurrence of these ringlets, for they sometimes were present in a sensory and sometimes in a motor column. The sudan stains were essentially negative and the Kulschitsky stains completely so. In the Spielmeyer preparations of these cords were present the same unstained patches noted in all the cords thus far described.

Ganglion cell changes in the ventral horns of the cord of a type seen in the animals of the two preceding groups, namely, loss of distinctness of cytoplasmic detail and deep staining, were present to a

slight degree in the animals of this group as well. No changes, however, were present in the nerve cells elsewhere than in the spinal cords. Each cerebral hemisphere, cerebellum, pons and medulla was completely normal as regards the cellular constituents, the myelin sheaths and the axis cylinders.

*Normal Controls.*—The peripheral nerves were completely negative as far as evidences of a genuine demyelination were concerned. In the Marchi preparations only was an occasional black granule seen within a neurilemma. Finding such solitary granules of degenerated myelin

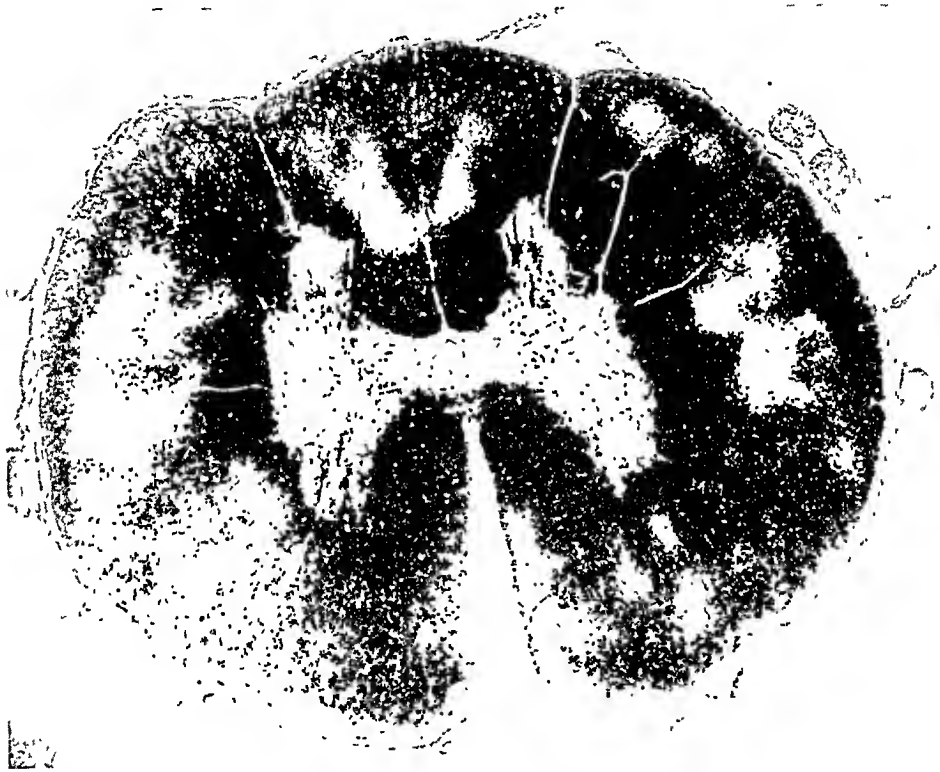


Fig. 13.—Photomicrograph of spinal cord of dog 10 stained by the Spielmeyer method. Note the unstained zones (cf. fig. 7);  $\times 15$ .

in what were quite obviously normal nerves would indicate that they were without pathologic significance. An occasional degenerated myelin sheath was also found in the preparations of the spinal cord treated with osmic acid, and was likewise, quite probably, without significance. The Kulschitsky stains and the sudan III stains of the spinal cords were completely negative, but in the Spielmeyer preparations patchy, unstained zones were present rather conspicuously (fig. 13). And these in the cords of normal control animals that at no time displayed neurologic manifestations!

An occasional anterior horn motor cell was deeply stained and lacked a well defined Nissl apparatus. From this it must be concluded that findings of a similar nature in all the animals previously described were of minimal, if any, significance. No lesions of any kind were demonstrable in the cerebral hemispheres, the brain stem and the cerebellum by any of the histologic methods employed.

*Summary of Anatomic Findings.*—In none of the twelve animals studied was there any gross evidence of disease in the central and peripheral nervous systems. Microscopically, a peripheral polyneuritis, in the sense of myelin degeneration, was found in the sciatic, median and ulnar nerves, the brachial plexuses and the vagus nerves, decreasing in severity in the order named, and involving all the animals except the two normal controls (dogs 9 and 10). The neuritis implicated the bilateral nerves to an equal degree, was as marked in the proximal as in the distal portions of each nerve, and appeared to parallel in severity the duration of the clinical symptoms, except in the starvation controls (dogs 11 and 12), which did not develop paralytic manifestations. In the latter two animals the neuritis, though definitely present, was decidedly less marked than in the animals maintained on a vitamin B-deficient ration. The involvement of the axis cylinders was trivial, even in those nerves in which the myelin destruction was severe.

In none of the animals were changes found in the spinal nerve roots. Tigrolysis, pigmentation and acute swelling were present in an occasional anterior horn motor cell in the spinal cords of all the animals. An occasional black ringlet of myelin degeneration was found by the Marchi method in the experimental animals, as well as in the controls. Sudan III preparations of the spinal cord were invariably negative, as were the modified Weigert preparations except in dog 1. In this animal (which was on a highly purified diet during the whole course of the experiment), there was degeneration of the fasciculus gracilis demonstrable not only in the Kulschitsky preparations, but in the Marchi and Nissl preparations as well. In the latter stains, it was seen that the median dorsal fasciculi contained numerous regressive glia—microglia and myeloclasts.

In the Spielmeyer preparations of all the spinal cords, of the normal controls, the fasting controls and the animals on vitamin B-deficient diet, there were found disseminated, large, irregular, unstained areas involving motor as well as sensory tracts. In serial sections stained by this method, it was noted that the unstained patches in no two consecutive preparations occurred in precisely the same location.

In dogs 1, 5 and 6, which were either entirely or partially on the purified casein diet (table 2), there were found large zones of degeneration and vascular proliferation in the vermis of the cerebellum and



the regions of the substantia gelatinosa rolandi of the pons and medulla. These lesions were present only in the three dogs enumerated and were strikingly similar as regards the type, the location and the bilateralism (substantia gelatinosa) of the involvement.

The remainder of the central nervous system revealed no pathologic changes by any of the methods of study employed in these experiments, excepting dog 4. In the cerebral cortex of the latter animal, which had unusual epileptiform seizures in addition to the more usual paralytic symptoms, there were found disseminated ischemic necrobiotic foci in the Nissl preparations. These lesions were not associated with any demonstrable organic vascular changes.

#### COMMENT

From these studies on the dog the anatomic findings resulting from lack of the antineuritic factor in pigeons and rats have been confirmed as far as concerns a noninflammatory peripheral polyneuritis. Further, the fact is substantiated that even with very severe lesions of the medullary sheaths the axis cylinders of the peripheral nerves suffer a minimal degree of damage. A finding of decided interest, however, is the polyneuritis observed in the dogs (nos. 11 and 12) that were totally deprived of food except for the calculated daily requirement of the vitamin B complex. Mention has been made in an earlier part of this paper of similar observations by Woollard<sup>8</sup> on the rat, but he noted, as did we in the dog, that in these animals the myelin destruction was not as marked as in those that received a vitamin B-deficient diet. Neither the dogs nor the rats at any time displayed signs of a neuromuscular lesion clinically, and therefore, on first thought, it would appear that a polyneuritis could not be held responsible for the neurologic syndrome observed in the vitamin B-deficient animals. Only it must be noted that death occurs much sooner in the completely starved dogs (fifteen and thirty-five days, respectively, in the two animals of this study) than in those maintained on a vitamin B-deficient ration. Is it not possible that inanition in itself is capable of producing the observed polyneuritis, but that early death interferes with the onset of the clinical symptoms of paralysis and ataxia? The less severe anatomic lesion in the peripheral nerves of the starved controls, as contrasted with that in the vitamin B-deficient animals, would tend to substantiate this hypothesis.

To attempt to explain all the neurologic signs on the basis of a polyneuritis is unwarranted, particularly the convulsions and opisthotonos. A great variety of lesions—in the anterior horn motor cells of the spinal cord, in the medullary sheaths of the spinal cord and cerebrum and even in the cerebral cortical ganglion cells—are held



responsible for these clinical signs by a variety of workers. Lesions of such nature, namely, chromatolysis, pigmentation and shrinkage of the anterior horn motor cells and degeneration of an occasional myelin sheath in the spinal cord, have been observed in this study, but never to a degree greater than in the normal control animals. It would appear, therefore, that they play no rôle in the pathogenesis of these nervous symptoms.

In this connection, the findings in dog 4 are of especial interest. This animal had epileptiform seizures with foaming at the mouth and marked retractions of the head. At necropsy, the cerebral findings were identical in many respects with those reported by Spielmeyer<sup>18</sup> and by DeVries<sup>19</sup> as the result of functional vascular spasms. Cortical necrobiotic foci occur, particularly in the cornu ammonis formation, in certain forms of epilepsy, and in other parts of the cerebral cortex in such diverse conditions as manic depressive psychosis, carbon monoxide poisoning, eclampsia and uremia. In many such instances, no organic vascular lesions are demonstrable, and it is held that ischemia on a functional basis is the underlying cause of these anatomic alterations. Not all cases of epilepsy, however, show necrobiosis, and it is believed that fairly prolonged and repeated attacks of functional vascular occlusion are necessary before these changes take place. It is at least of interest to speculate whether some of the manifestations observed in B-avitaminosis are not due to just such causes, namely, functional vascular disturbances in the central nervous system.

The work of Gildea, Kattwinkel and Castle<sup>12</sup> already referred to, on a combined system disease in the spinal cord, portrays lesions by the Spielmeyer technic identical with those described in this study. From the facts that these large patchy unstained zones in the cord were demonstrable only by the Spielmeyer method, that they failed to occur in the same location in any two consecutive preparations, and that they were found in the normal controls as well as in the fasting controls and vitamin B-deficient animals, it must be concluded that they represented merely artefacts produced by the staining technic. Artefacts of this nature are not infrequently encountered in this method even in human material when the various steps in staining are not scrupulously adhered to, particularly as regards washing in 70 per cent alcohol following the "iron alum mordant."

It is to be noted that the three animals subjected completely or in part to deprivation of the pellagra-preventive factor (B<sub>2</sub>) displayed a multiplicity of anatomic changes in addition to the peripheral poly-

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18. Spielmeyer, W.: *Kreislaufstörungen und Psychosen*, Ztschr. f. d. ges. Neurol. u. Psychiat. **123**:536, 1930.

19. DeVries, E.: *Acute Diseases of the Brain Due to Functional Disturbance of the Circulation*, Arch. Neurol. & Psychiat. **25**:227, 1931.

neuritis. One such change was the demyelination of the median dorsal columns in dog 1, a finding similar to that described by Winkelman<sup>20</sup> in one of four cases of human pellagra. In a study of seven cases of human pellagra with nervous manifestations, Pentschew<sup>21</sup> found sclerotic changes in the spinal cords in four instances. These lesions, however, were not solely confined to the dorsal columns and this worker concluded that the neurologic changes in pellagra cannot be characterized as a "system disease."

Pentschew described in the first case of his series an atrophy and sclerosis of one of the cerebellar lobules, and in the fourth case an "inflammatory and degenerative" lesion in the locus caeruleus of the pons. In this connection, it is interesting to bear in mind the lesions of all three animals that were deficient in the B<sub>2</sub> factor. Whether the findings observed in Pentschew's cases and those in our three animals are, however, more than coincidental must remain a mooted question.

Pappenheimer and Goettsch<sup>22</sup> recently described softenings in the cerebellum in chicks, which they attributed to a lack of vitamin E. They do state, however, that their diets may not have been adequate as regards the water-soluble B vitamins. It is interesting that these lesions in the cerebellum in chicks are in every way identical with the cerebellar lesions present in the vitamin B<sub>2</sub> deficient dogs of our study.

#### CONCLUSIONS

Extensive demyelination was present in the sciatic, median, ulnar and vagus nerves and in the brachial plexuses of dogs deprived of the water-soluble B vitamins.

This myelin destruction was most severe in the sciatic and least severe in the vagus nerves of all the animals subsisting on a vitamin B-deficient ration.

The extent of the anatomic alterations in the peripheral nerves varied in direct proportion to the severity and duration of the paralytic symptoms.

Peripheral polyneuritis of the same type, but of a less degree, was present in the control animals, which were entirely deprived of food but supplied with an adequate amount of the B complex.

Ganglion cell changes and disseminated foci of myelin destruction in the brain or spinal cord could not be held responsible for the clinical symptoms of this nutritional disorder.

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20. Winkelman, N. W.: Beiträge zur Neurohistopathologie der Pellagra, *Ztschr. f. d. ges. Neurol. u. Psychiat.* **102**:38, 1926.

21. Pentschew, A.: Ueber die Histopathologie des Zentralnervensystems bei der Psychosis pellagrosa, *Ztschr. f. d. ges. Neurol. u. Psychiat.* **118**:17, 1928.

22. Pappenheimer, A. M., and Goettsch, M.: A Cerebellar Disorder in Chicks, Apparently of Nutritional Origin, *J. Exper. Med.* **53**:11, 1931.

Dogs deprived wholly or in part of the thermostable B<sub>2</sub> factor showed large zones of degeneration and vascular proliferation in the vermis of the cerebellum and in the region of the substantia gelatinosa rolandi.

The dog that had subsisted throughout the entire experimental period on the highly purified ration deficient only in the heat-stable factor (B<sub>2</sub>) evidenced degeneration in the fasciculus gracilis of the spinal cord.

#### PROTOCOLS

*Dog 1.*—A female, weighing 9 Kg., subsisted on the highly purified casein diet supplemented with subminimal amounts of antineuritic vitamin B (table 2). The first appearance of symptoms occurred on the fifty-seventh day. The syndrome was exhibited in its most acute aspects. Both hind and fore limbs were severely paralyzed; convulsions were frequent, with marked opisthotonos. The body temperature was 101 F. Bloody diarrhea was present. The animal died within sixteen hours after the onset of the symptoms. Its final weight was 6.8 Kg.

Examination was performed one hour post mortem. The positive observations included dilatation of all four cavities of the heart, somewhat enlarged adrenals, several erosions of the mucous membrane in the ileum and larger ulcerations in the rectum. The buccal and gingival mucous membranes were unaltered.

*Dog 2.*—A male, with an initial weight of 10.6 Kg., subsisted on casein III. After a three weeks' period of perfect appetite, the intake of food became erratic until the fortieth day, after which anorexia was broken by only a few intermittent days of partial ingestion of food. The first signs of antineuritic vitamin deficiency were manifested on the sixty-eighth day. Until the seventy-fourth day, characteristic convulsions occurred with progressively greater frequency. The animal was killed the following morning. Its final weight was 7.5 Kg.

Examination was made three hours post mortem. The entire body, except the nervous system, was without abnormalities.

*Dog 3.*—A male, weighing 10.6 Kg., subsisted on casein III. The animal ate fairly well throughout the period, although the intake of food was erratic in the latter half of the experiment. On the seventy-first day, a maintained contracture of the hind legs was observed. By the seventy-fourth day, the dog exhibited a pronounced ataxia when allowed to walk around the room. During the afternoon of the same day, the animal became hypersensitive to tactile stimulation, with the consequent occurrence of tonic and clonic spasms, marked opisthotonos being present. The convulsions occurred more and more frequently until, on the seventy-fifth day, the animal was killed with a lethal dose of iso-amyl-ethyl barbituric acid. Its final weight was 9.6 Kg.

Examination was made one hour and fifteen minutes post mortem. The positive findings included a large subepicardial hemorrhage over the anterior surface of the left ventricle. A section of the myocardium revealed fibrosis and a recent hemorrhage in the musculature of the left ventricle. The adrenals and the pancreas were of normal size and texture. The intestinal mucosa was pale and intact.

*Dog 4.*—A female, with an initial weight of 18.3 Kg., subsisted on casein III. After two weeks of perfect intake of food, complete anorexia persisted until the end of the experimental period. On the forty-fourth day, an epileptiform convulsion occurred, after which the animal did not exhibit any more signs of antineuritic vitamin deficiency until the fifty-first day. On this day, three such convulsions were observed, involving the facial muscles as well as the body

musculature. The animal frothed at the mouth during the spasms. Throughout the subsequent morning, severe, generalized convulsions occurred in rapid succession. The dog died at 3:30 p. m. on the fifty-second day. Its final weight was 12.4 Kg.

Necropsy was performed three hours post mortem. There were hypertrophy and dilatation of the right cardiac ventricle. The adrenals were normal in size, color and consistency. There were worms in the small intestine, but there was no ulceration of the mucosa. The gums were normal.

*Dog 5.*—A male, weighing 6.5 Kg., had subsisted on the highly purified casein diet supplemented with antineuritic vitamin B, but deficient in vitamin B<sub>2</sub>. After receiving a large dose of whole yeast, the animal was transferred to casein III, the antineuritic substance (B<sub>1</sub>) being withheld. After a week of perfect appetite, the intake of food became erratic until the twenty-seventh day, following which complete anorexia ensued. The animal vomited on the fortieth day. On the forty-first day, spasticity of the hind legs was apparent. By the forty-second day, the dog could not rise to its feet, and on the subsequent day it remained in an opisthotonic position, with maintained extension of all limbs. A bloody diarrhea was present. The animal died at 12 noon. Its final weight was 4.9 Kg.

Necropsy was performed one hour post mortem. The heart was flaccid and dilated. There was focal pneumonia in the right lung on gross and microscopic examination. The adrenals were normal. Numerous hemorrhages and marked congestion were seen in the intestinal mucosa, but no ulceration. There were hair balls in the stomach and colon. The gingivae were normal.

*Dog 6.*—A male, with an initial weight of 5.9 Kg., had subsisted on the purified casein diet supplemented with vitamin B<sub>1</sub>, but deficient in the B<sub>2</sub> substance. It was given a large dose of whole yeast and transferred to casein III. On the forty-third day, the animal vomited and exhibited a marked incoordination of gait. Bloody diarrhea was observed. The dog died on the forty-fourth day with typical symptoms. Its final weight was 8.4 Kg.

Examination was made thirty minutes post mortem. The heart was normal. There was focal pneumonia in the right lung. The duodenal mucosa had several eroded zones about 1 cm. in greatest diameter from which there was bleeding into the duodenal lumen. There were numerous superficial ulcers, the size and shape of solitary lymph follicles and Peyer's patches, in the large and small intestine. There was frank hemorrhage in the ileum. The adrenals were grossly and microscopically normal. There were no changes in the gums.

*Dog 7.*—A male, weighing 9.9 Kg., subsisted on casein III. After sixteen days of perfect appetite, the intake of food was erratic until death. On the thirty-seventh day, an acute onset of the symptoms characteristic of antineuritic vitamin deficiency occurred. Spastic paralysis of the limbs progressed cephalad very rapidly, and convulsions recurred frequently. Marked opisthotonos was observed. The animal died at 9 p. m. the same day. Its final weight was 8.4 Kg.

Examination was made two hours post mortem. All four ventricles of the heart were dilated, but without cardiac hypertrophy. Worms were present in the ileum, as well as an occasional small mucosal erosion. No abnormalities were noted in the adrenals or in the gums.

*Dog 8.*—An old female, weighing 12.4 Kg., after ninety days of subsistence on the casein III ration, was observed to eat its feces; in this way, it was securing some of the missing essential daily and thereby delaying the development of symptoms. The animal was muzzled. On the one hundred and sixth day, the symptoms of vitamin B<sub>1</sub> deficiency were manifested as a persistent extension of the hind

legs and an incoordination of gait. The symptoms became more severe during the three subsequent days, convulsions occurring very frequently on the one hundred and eighth day. The animal died the following morning. Its final weight was 10 Kg.

Necropsy was performed four hours post mortem. Flame-shaped hemorrhages were seen in ventricular endocardium and in the myocardium. There were no ulcers in the intestines; there were no lesions in the adrenals or in the gums.

*Dog 9.*—A large normal male subsisted on a ration employed for stock animals and found adequate in all essentials as far as known. At no time did it present nervous manifestations.

It was killed and examined immediately. The results were negative.

*Dog 10.*—A large male with a Pavlov pouch used for studies on gastric secretion was killed with iso-amyl-ethyl barbituric acid and examined immediately. The results of the entire postmortem examination were negative.

*Dog 11.*—From a male, weighing 5 Kg., food was completely withheld except 1 Gm. of vitavose per kilogram per day to supply a sufficient amount of the B complex. Water ad libitum was allowed. The animal died fifteen days from the beginning of the fast. No paralytic symptoms were observed.

Necropsy was performed two hours and forty-five minutes post mortem. There were no positive findings except marked emaciation and changes in the nervous system.

*Dog 12.*—A female, weighing 6 Kg., was starved completely except for 1 Gm. of vitavose per kilogram per day. The intake of water was not restricted. The animal died thirty-five days from the beginning of its fast.

Necropsy was performed four hours post mortem. The results were completely negative except for marked emaciation and changes in the nervous system.

# RENAL DENERVATION

THE EFFECT OF SNAKE VENOM AND CHILLING ON THE  
RENAL VASCULARIZATION \*

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In the case history of the nephritic patient one clinical observation has been established as common—the “chill,” or at least the exposure to cold. Of course, we may not find it in the history of each patient, but it occurs with such frequency that etiologic significance has always been attached to it.

It is known that following chilling the urine of presumably normal persons may occasionally contain albumin, some red blood cells and casts; in persons with vasomotor lability, this occurs rather frequently.

The fact that under normal conditions the vascular bed of the kidney receives a proportionally large share of the total circulating blood is also well established, as is the fact that ischemia is followed by obvious functional and histologic evidence of renal degeneration, if the condition obtains for any length of time (thirty minutes or more).

In previous papers,<sup>1</sup> we have shown the intimate autonomic coordination that exists between the vascular bed of the skin and the internal organs; when chilling occurs, the vascular bed of the splanchnic area dilates (stomach, intestines, liver, pancreas and spleen), but the renal vessels apparently contract synchronously with the skin.

We have repeatedly called attention to the significance of the state of the vascular bed of an organ in relation to resistance against injury and infection,<sup>2</sup> a factor that is of particular importance in organs with great metabolic demands (such as the kidney) where vascular spasm may become a factor of paramount interest.

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\* From the Department of Pathology, University of Illinois College of Medicine, Chicago, and the Medical Department, University of Hamburg, Hamburg.

1. Petersen, W. F., and Müller, E. F.: The Splanchno-Peripheral Balance, *Arch. Int. Med.* **40**:575, 1927.

2. Müller, E. F., and Petersen, W. F.: Ueber den Infektionsschutz des Lebergewebes bei experimenteller Sepsis, *Ztschr. f. exper. Path. u. Therap.* **66**: 442, 1929.

As a matter of fact, the significance of arteriolar spasm in the pathogenesis of renal disease has been repeatedly suggested, particularly in the arteriosclerotic types of nephritis.

Some years ago, when studying the effects of prolonged continuous intravenous injections of *Bacillus coli* (whereby the typical picture of a sepsis could be obtained), we observed<sup>3</sup> the rapid appearance of albumin, red blood cells and casts in the urine in association with the onset of the chill in the animal. We then proceeded to a study of such urinary changes when the kidney had been denervated.

In these experiments, we found that when *B. coli* was injected intravenously over long periods of time, the urine from the denervated kidney remained free from albumin, red blood cells or casts, while the urine from the normal kidney contained them from the time of the first chill.<sup>4</sup> The urine from the denervated kidney remained free from the bacteria and that from the normal kidney contained them in large numbers.

A latent period of approximately half an hour usually followed the injection of the bacteria into dogs before the onset of a chill. We therefore proceeded next to chill the animals artificially and simultaneously with the beginning of the injection. It was then found that the urine from the denervated kidney remained normal, while the urine from the normal kidney contained red blood cells, casts, albumin and bacteria from the very beginning of the injection.<sup>5</sup>

From these considerations and observations we felt justified in implicating both the vasomotor status and the varying toxic agent in the production of the nephritic picture.

#### RENAL INNERVATION

The innervation of the kidney was reviewed by Kuntz,<sup>6</sup> who concluded that the nerves are vasomotor and chiefly vasoconstrictor. Kaufmann and Gottlieb<sup>7</sup> demonstrated nerve fibers to the tubules, to

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3. Petersen, W. F.; Milles, G., and Müller, E. F.: Ueber Aenderungen des Kalium—Calcium Quotienten der Lymphe bei experimenteller Sepsis, *Ztschr. f. exper. Path. u. Therap.* **60**:336, 1928.

4. Müller, E. F.; Petersen, W. F., and Rieder, W.: Die Bedeutung des vegetativen Systems für die Entstehung der primären Nierenschädigungen im Anschluss an Erkältungen und Infektionen, *Verhandl. d. deutsch. Gesellsch. f. inn. Med.* **42**:580, 1930.

5. Footnote 5 deleted by the author.

6. Kuntz, A.: *The Autonomic Nervous System*, Philadelphia, Lea & Febiger, 1929, chap. 12, p. 271.

7. Kaufmann, J., and Gottlieb, R.: Innervation of Renal Parenchyma; Study to Demonstrate Nerve Endings in Renal Epithelium, *Am. J. Physiol.* **96**:40, 1931.

which they ascribed a secretory function. An autonomic sensory function may further be inferred from the successful relief of renal pain by denervation, as demonstrated by S. H. and R. G. S. Harris<sup>8</sup> and others. Ellinger and Hirth<sup>9</sup> presented evidence for a somewhat more complicated nervous function in the form of selective excretion influenced by various elements in the renal nerve supply. Studies of the renal function following denervation in the normal animal were made by Milliken and Kare,<sup>10</sup> de Gironcoli,<sup>11</sup> Caldwell, Marks and Rowntree,<sup>12</sup> Seres<sup>13</sup> and many others. All, except Seres, agreed that denervation results in at least a temporary increase in renal activity, followed by a return to a normal state in from two to five months. Seres stated that renal denervation is followed by renal insufficiency. This conclusion, however, is adequately contradicted by the other workers. S. H. and R. G. S. Harris performed renal denervation in twenty-eight patients, with no ill effects. We have not seen any evidence of injury to the kidney in observations on dogs lasting over a period of eight months. Extensive histologic and functional studies on denervated kidneys have been published. There is, however, little or no information available concerning the response of the denervated kidney to various insults as compared to the intact kidney, especially of the vascular bed under such conditions, and we therefore present the results of the experiments detailed in the following pages with this object in mind. Milliken and Kare noted that the function of the normal kidney is inhibited by ether anesthesia while that of the denervated kidney is unaffected. S. H. and R. G. S. Harris performed a bilateral denervation in one case of essential hematuria and in a second case of parenchymatous nephritis, with reported satisfactory results.

#### DENERVATION

Dogs were used throughout, and all operative procedures were performed under ethyl barbiturate anesthesia (Nembutal-Abbott).

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8. Harris, S. H., and Harris, R. G. S.: Renal Sympathetico-Tonus and Renal Sympathectomy, *Canad. M. A. J.* **24**:235, 1931.

9. Ellinger, P., and Hirth, A.: Function of the Renal Nerves, *Arch. f. exper. Path. u. Pharmacol.* **106**:135, 1925.

10. Milliken, L. F., and Kare, U. G.: The Influence of the Nerves on Kidney Function in Relation to the Problem of Renal Sympathectomy, *J. Urol.* **13**:1, 1925.

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12. Caldwell, J. M.; Marks, H., and Rowntree, L. G.: Renal Function After Bilateral Denervation of the Kidney in Normal Dogs, *J. Urol.* **25**:351, 1931.

13. Seres, M.: Denervation of the Kidney, *Rev. méd. de Barcelona* **1**:220, 1925; abstr., *Ztschr. f. urol. Chir.* **17**:54, 1925.



Unilateral denervation (left) was performed through an infracostal incision. The kidney was delivered through the wound, the peritoneum was divided, and the fat and the areolar tissue about the hilus were stripped away. Usually several nerves could be demonstrated grossly, and these were divided. The perivascular sheath was stripped down, and the artery was further deprived of its externa by rubbing between the blades of the hemostat. The kidney was returned to its normal position and the wound closed. Recovery to normal was then permitted for several weeks before experiments were made.

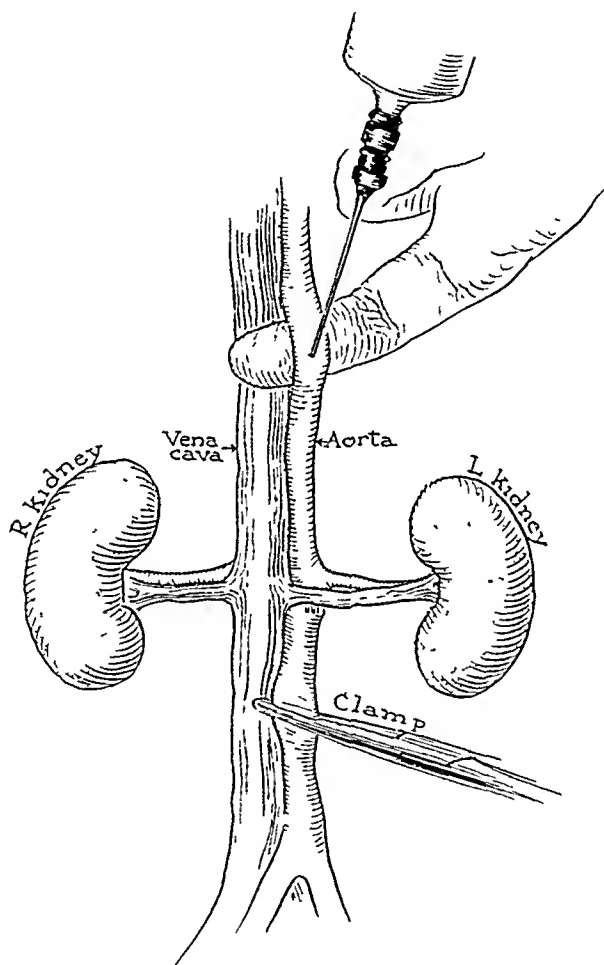


Fig. 1.—A diagram indicating the point of injection into the aorta relative to the position of the renal arteries.

#### ROENTGENOGRAPHIC DEMONSTRATION OF VASCULAR BED

A 25 per cent suspension of bismuth oxychloride in water was injected into the vascular bed of the kidney. It has been our experience that this mixture gives better results in antemortem injection than the acacia-bismuth oxychloride mixture recommended by Hill<sup>14</sup> for

14. Hill, E. C.: A Radio Opaque Bismuth Suspension for Anatomical, Histological and Pathological Research, *Bull. Johns Hopkins Hosp.* **44**:248, 1929.

postmortem injection. The technic of the injection was as follows (fig. 1):

The aorta was exposed through a left paravertebral incision. In order to obtain a good exposure, the last rib was removed, and occasionally the crus of the diaphragm was divided. The aorta was occluded below the origin of the renal vessels, and 50 cc. of a 25 per cent suspension of bismuth oxychloride was injected into the aorta above the origin of the renal arteries. The kidneys were removed from three to five minutes after the injection and bisected, and roentgenograms were made, a low voltage (60 volts) and a low milliamperage (5 milliamperes) being used, with an exposure of from four to five seconds.

#### THE VASCULAR BED IN THE DENERVATED KIDNEY

In normal kidneys in normal animals, the pictures obtained of the vascular bed are fairly uniform, as indicated in figure 2. It will be

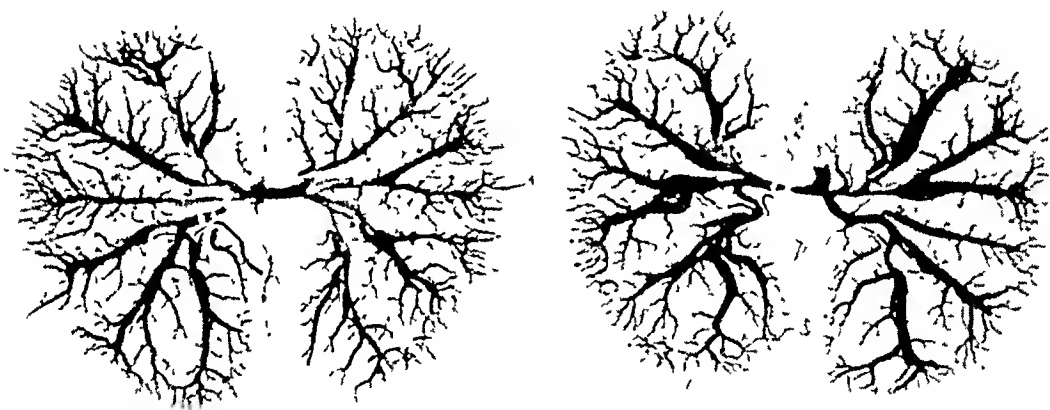


Fig. 2.—The renal vascular beds of a young normal dog after the injection of bismuth. Note the uniformity of extent and distribution of the arcuate and interlobular blood vessels.

noted that while there is some slight difference in the degree of injection shown by the large blood vessels on the two sides, the arcuate arteries and the arteria recti show a uniform and equal injection of bismuth for a distance of about three quarters of the width of the cortex. It will be further noted that the outlines of the large vessels are regular.

In some instances there may be but little filling of the large vessels with the material injected, although here again the fine vessels show a uniform and equal injection on the two sides.

In contrast with this uniform injection into the vascular tree in the normal kidney, a distinct increase in the size and number of blood vessels showing injection is apparent in pictures obtained from denervated

kidneys. This change in the vascularity is apparent as early as forty-eight hours and persists for an indefinite period (lasting at least two months). The experiments described in the following paragraphs are typical of the series.

*Dog 1* (fig. 3).—The left kidney was denervated on April 4, 1930, and bismuth was injected into the renal vascular bed and roentgenograms were made two days later. The marked increase in the vascular bed demonstrated is associated with an increase in the size of the denervated (left) kidney. In microscopic sections, the only apparent difference is an increase in the size and the number of blood vessels and in the amount of contained blood in the denervated (left) kidney.

*Dog 2* (fig. 4).—The left kidney was denervated on June 23, 1930, and bismuth was injected into the renal vascular bed eighteen days later. In spite of injury



Fig 3 (dog 1).—The left kidney was denervated forty-eight hours prior to the injection of bismuth into the vascular beds. Note the general vascular dilatation and particularly the large size of the arteria recti and their more marked projection to the margin of the cortex.

to the kidney in the course of the operation, the vascular bed was more widely dilated and the blood vessels were more abundantly demonstrated in the left kidney than in the right. There was no difference in the size of the two kidneys. These differences were especially well demonstrated in a thin section of kidney as seen in figure 6. The microscopic picture corresponded closely to that seen in the previous animal.

*Dog 3* (fig. 5).—Dog 3 was old, being probably 8 or 9 years of age. The kidneys were grossly scarred and firm in consistency. The left kidney was denervated on Aug. 10, 1930. Bismuth was injected into the renal vascular bed two months later. The left kidney had increased considerably in size, weighing 42 Gm.; the right weighed but 30 Gm. The vascular beds of both kidneys showed evidence of serious damage, but that in the left was obviously more widely open

than that in the right. In microscopic sections of the right kidney there were areas, corresponding to the depressed scars on the surface, revealing the glomeruli atrophied, disintegrating or obliterated, with Bowman's space widely dilated and containing more or less clear, homogeneous exudate. In these areas, the tubules were poorly demarcated; their epithelium was shrunken and granular or entirely

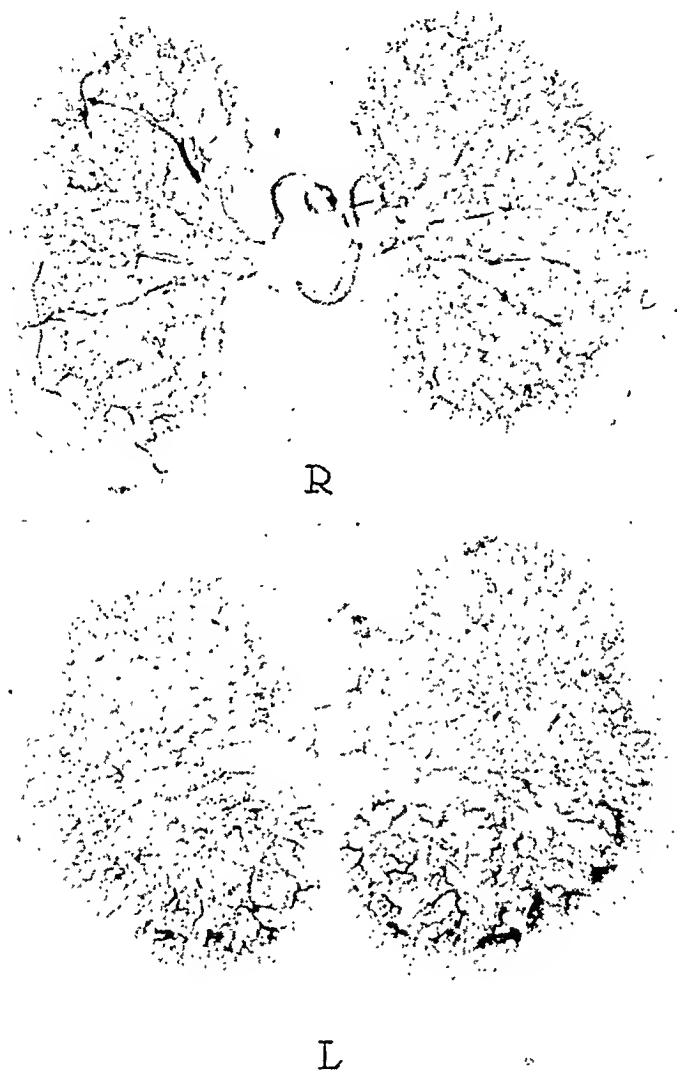


Fig. 4 (dog 2).—The left kidney was denervated on June 23, 1930. The roentgenogram shows the renal vascular beds eighteen days later, after the injection of bismuth.

lost. The lumina were small, and to a considerable extent the tubules were replaced by interstitial fibrous tissue. There was a diffuse interstitial round cell infiltration. Between these scarred areas the glomeruli were seen. The tubules were widely dilated, the epithelium was granular and shrunken, and a small amount of granular debris was present in the lumina. The blood vessels were of normal

dimensions, and the larger ones were well filled with the injected material. The left kidney presented the same appearance in the scarred areas, but in the intervening areas the glomeruli and tubules were much better preserved, and more injected material was seen in the finer vessels.

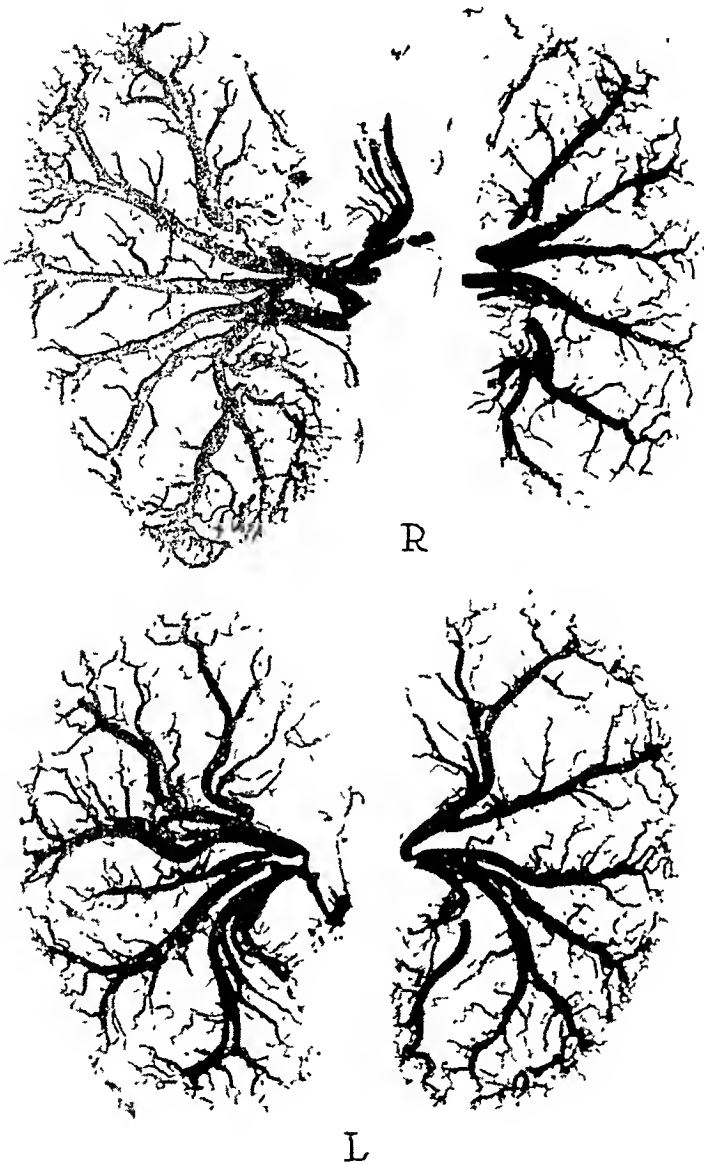


Fig. 5 (dog 3).—The left kidney was denervated on Aug. 10, 1930. The roentgenogram shows the renal vascular beds sixty-one days later, after the injection of bismuth.

*Dog 4.*—The left kidney was denervated on June 4, 1930. On August 20, 10 Gm. of iopax was injected intravenously, giving a shadow of the left pelvis only. The injection of bismuth into the kidney was unsatisfactory, but it nevertheless demonstrated a vascular bed rather wider open in the left kidney. The left kidney weighed 90 Gm.; the right, 65 Gm. Microscopically, the glomeruli of the

right kidney were of moderate size; the capillary loops were somewhat dilated and contained a few granules of injected bismuth but little blood. The tubular epithelium was rather granular, and the nuclei were pale. The blood vessels contained a moderate amount of bismuth and few blood cells. The glomeruli of the left kidney were larger than those of the right, and the dilated capillary tufts, as well as the interstitial blood vessels, were distended with red cells. The tubular epithelial cells showed rather more evidences of degenerative changes, and hyaline plugs were numerous in the lumina.

#### EFFECT OF EPINEPHRINE

A single injection of epinephrine was made into each of several normal and denervated dogs, and in each case bismuth was injected into the renal vascular bed in order to compare, in the vascular spasm obtained, the effectiveness of the denervation.

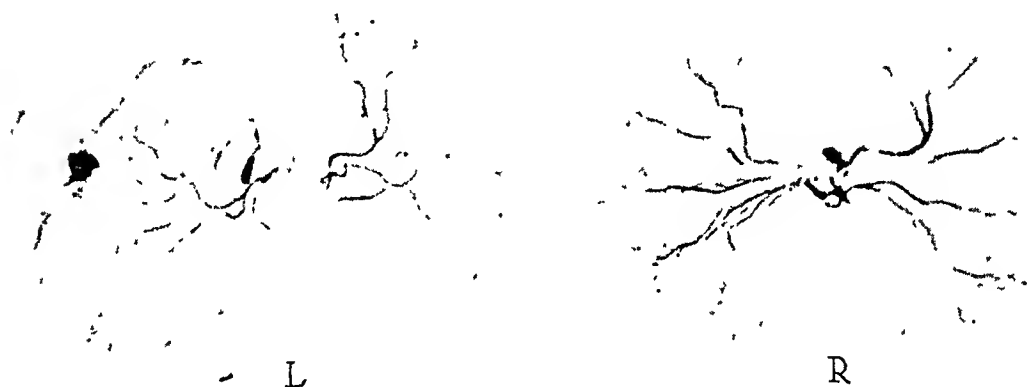


Fig. 6 (dog 5).—The vascular beds of normal kidneys after 2 cc. of a 1:10,000 dilution of epinephrine had been injected into the left renal artery followed by the injection of bismuth into both kidneys a few minutes later.

*Dog 5 (fig. 6).*—Two cubic centimeters of a 1:10,000 dilution of epinephrine was injected into the left renal artery of a young, normal dog. Very quickly general systemic effects were noted, and bismuth was injected into the kidneys. Grossly both kidneys were pale. The roentgenogram demonstrated obliteration of the small and medium-sized blood vessels in the left kidney, with a less marked effect in the right kidney. Microscopically, the glomeruli in the right kidney were uniformly distended, their capillary tufts were filled with erythrocytes, and bismuth granules were seen in many. The tubular epithelium was slightly granular. The blood vessels were distended with cells and bismuth.

In the left kidney, the glomerular capillaries contained much less blood and no granules of bismuth, and the blood vessels of the parenchyma contained few erythrocytes and little bismuth.

The roentgenogram of the vascular bed into which bismuth had been injected, as well as the histologic picture, showed the local action of epinephrine to be on the blood vessels.

*Dog 6* (fig. 7).—The left kidney of dog 6 was denervated on Sept. 16, 1930. On March 30, 1931, 5 cc. of a 1:10,000 dilution of epinephrine was injected into the aorta above the origin of the renal vessels. Three minutes later, bismuth was injected into the kidneys. In the right kidney, there was almost complete obliteration of the finer blood vessels. In the left kidney (with the exception of a few patches in which the finer vessels were obliterated), a practically normal vascular bed was found. In the microscopic section of the right kidney, the glomeruli were constricted and contained little blood, and few interstitial blood vessels were seen. The sections of the left kidney stood in marked contrast with this picture. The vessels were dilated and prominent, the glomeruli were large, their capillaries distended with blood, and much injected material had reached the glomeruli.

#### SNAKE VENOM

The effect of rattlesnake venom is that of a powerful vascular poison causing endothelial injury, vascular paralysis and interstitial

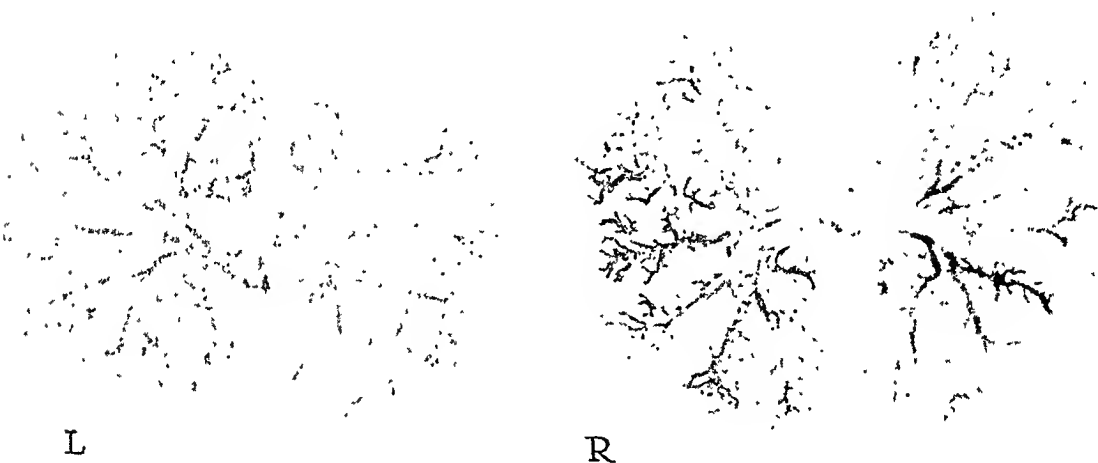


Fig. 7 (dog 6).—The left kidney was denervated on Sept. 16, 1930. Six and a half months later, 5 cc. of a 1:10,000 dilution of epinephrine was injected into the aorta above the origin of the renal arteries, followed a few minutes later by an injection of bismuth.

hemorrhages (Noguchi<sup>15</sup>). For this reason, we considered it of interest to determine the comparative effects of this material on the normal and the denervated kidney.

*Dog 7.*—Two tenths of a milligram of rattlesnake venom per kilogram of body weight was injected intravenously into a normal dog on Aug. 27, 1930, and bismuth was injected into the kidneys on Sept. 3, 1930. The roentgenogram demonstrated moderate beading of the large blood vessels; the small vessels were well preserved, and in the microscopic sections were seen moderate glomerular congestion and deeply stained, somewhat swollen tubular epithelium. The vessels were normal.

15. Noguchi, H.: An Investigation of Venomous Snakes with Special Reference to the Phenomena of Their Venoms, Washington, Carnegie Institute, 1903, vol. 141, p. 106.

*Dog 8* (fig. 8).—Into a normal dog, 0.5 mg. of rattlesnake venom per kilogram of body weight was injected intravenously on Sept. 8, 1930. On September 16, bismuth was injected into the renal blood vessels after the urine from the two sides had been collected separately over a period of six hours. The volume of urine secreted on the right was 25.8 cc., and that on the left, 30.5 cc. The ammonia nitrogen in milligrams per hundred cubic centimeters was 83 on the right and 85 on the left. The x-ray picture demonstrated marked beading of the large vessels of both kidneys, with almost complete obliteration of the fine vessels.

Microscopic sections displayed marked congestion of the glomeruli with here and there hemorrhage or exudation into Bowman's space and swelling of the

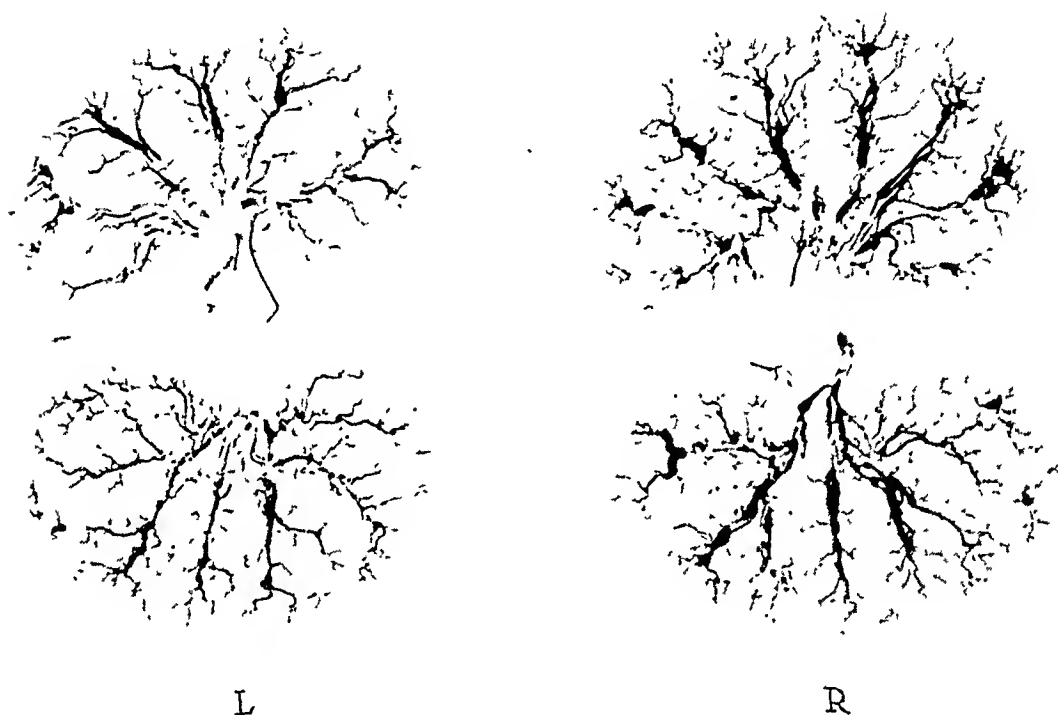


Fig. 8 (dog 8).—The vascular beds of intact kidneys eight days after the injection of 5 mg. of rattlesnake venom per kilogram of body weight, as delineated by an injection of bismuth on the eighth day.

capillary and arteriolar endothelium. The tubular epithelium was pale, swollen and granular, and the lumina contained much granular and hyaline debris.

*Dog 9.*—The left kidney was denervated on Sept. 19, 1930. On Dec. 23, 1930, 0.6 mg. of rattlesnake venom per kilogram of body weight was injected intravenously, and fifteen minutes later bismuth was injected into the kidneys. The entire left kidney showed an irregular injection of poor degree, with slight beading of the large blood vessels. The right kidney showed the large vessels well preserved, with a moderate decrease in the number of the small vessels that showed injection and in the degree of the injection.

Microscopically, the glomerular capillaries in the right kidney were moderately distended with blood, as were the interstitial capillaries and larger vessels. The



tubular epithelium was slightly swollen. Bismuth had penetrated to a few of the glomeruli and was found in small amounts in the larger vessels. In the left (denervated) kidney, the glomeruli were swollen and markedly congested, and their epithelial cells were swollen and pale. Here and there rupture with hemorrhages into Bowman's space was seen. The interstitial vessels were congested; the tubular epithelium was swollen and pale.

*Dog 10.*—The right kidney was denervated on Sept. 9, 1930. On Oct. 6, 1930, 1 mg. of rattlesnake venom per kilogram of body weight was injected intravenously. The dog died within twenty minutes, and bismuth was injected into the kidneys postmortem. The vascular beds in the two kidneys showed slight beading of the large blood vessels, with slightly less beading in the left. Microscopic sections, however, demonstrated more marked evidences of injury in the right kidney in the form of swelling of the tubular epithelium and marked congestion affecting particularly the glomeruli and interstitial blood spaces.

*Dog 11.*—The left kidney was denervated on Sept. 10, 1930. On Oct. 7, 1930, 0.5 mg. of rattlesnake venom per kilogram of body weight was injected intravenously. The dog went into profound shock, from which he recovered. On Oct. 8, 1930, the urine was collected separately from both kidneys for four hours. The left kidney secreted 19 cc. of urine, with a specific gravity of 1.036, and the excretion of ammonia nitrogen was at the rate of 38 mg. per hundred cubic centimeters. The right kidney in the same period secreted 13.5 cc. of urine, with a specific gravity of 1.038, and ammonia nitrogen at the rate of 29 mg. per hundred cubic centimeters. Bismuth was injected into the kidneys. The roentgenogram demonstrated a marked decrease in the finer vascular bed in the left (denervated) kidney, with a more mottled injection and some beading of the larger blood vessels. The right kidney displayed a practically normal vascular bed. Microscopically, the right kidney displayed a slight swelling of the glomerular endothelium, little blood in the capillaries, moderate interstitial edema and slight cloudy swelling of the tubular epithelium. Little injected material was seen in the smaller arterioles or glomeruli. In the left kidney, the glomerular endothelium was swollen; the capillaries were congested. The arteriolar endothelium was moderately swollen; the vessels contained considerable blood and bismuth. The tubular epithelium was the seat of marked cloudy swelling.

*Dog 12 (fig. 9).*—The left kidney was denervated on July 16, 1930. On Aug. 27, 1930, 0.5 mg. of rattlesnake venom per kilogram of body weight was injected, intravenously, and on Sept. 3, 1930, bismuth was injected into the renal vascular bed. The roentgenogram showed almost complete obliteration of the finer vascular bed of the left kidney with marked beading of the large vessels. In the right kidney, the finer vessels were well preserved and there was moderate beading of the larger vessels.

Microscopically, the glomeruli of the left kidney were markedly distended with blood, as were the interstitial blood spaces and blood vessels. The tubular epithelium displayed marked swelling in patchy areas, with deeply stained, granular appearing cytoplasm, and but little lumen was demonstrable. In the intervening areas, the tubular epithelium was vacuolated and broken down. In the right kidney, the evidences of injury were much less marked.

*Dog 13.*—The left kidney was denervated on July 16, 1930. On Aug. 20, 1930, 0.5 mg. of rattlesnake venom per kilogram of body weight was injected intravenously. On Sept. 5, 1930, bismuth was injected into the kidneys, and the roentgenograms demonstrated moderate diminution of the vascular bed of the left kidney, with slight beading of the larger blood vessels and a practically normal vascular bed of the right kidney.

*Dog 14.*—The left kidney was denervated on Sept. 5, 1930. Three days later 0.6 mg. of rattlesnake venom per kilogram of body weight was injected intravenously. This was followed by a severe reaction with marked vomiting. On Sept. 18, 1930, bismuth was injected into the kidneys. The larger blood vessels showed marked beading. There was a somewhat better injection into the fine vessels on the left than on the right.

*Dog 15.*—The left kidney was denervated on May 27, 1930. Five months later, 1 mg. of rattlesnake venom was injected intravenously, and after a period of twenty minutes bismuth was injected into the renal vascular bed. The filling of the blood vessels of the left kidney was demonstrated in the roentgenogram to be poor and patchy; the vessels were narrow and constricted. In the right

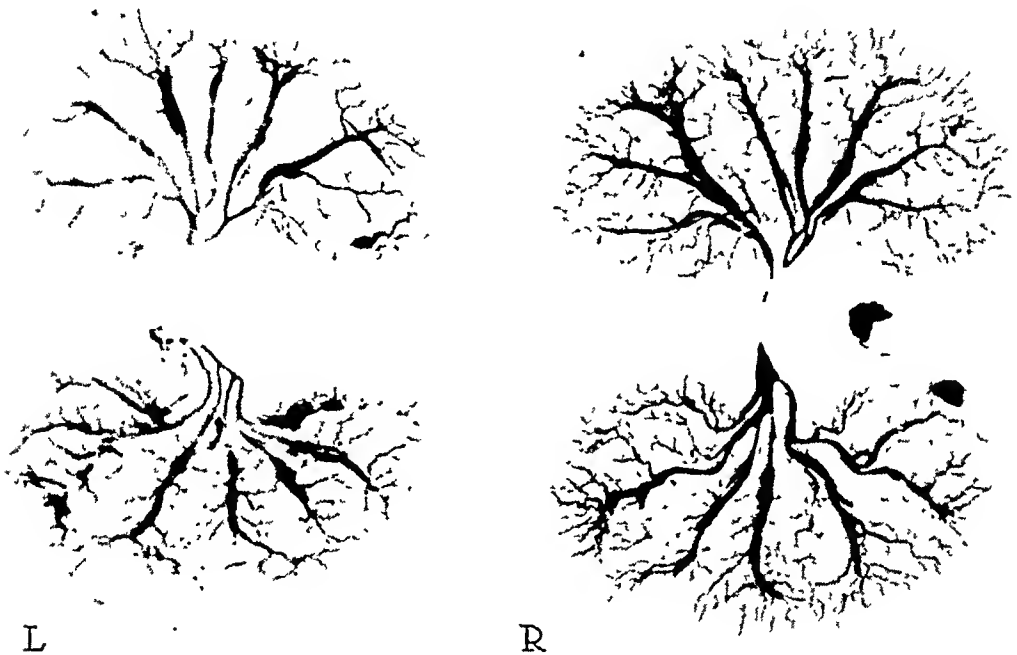


Fig. 9 (dog 12).—The left kidney was denervated on July 16, 1930. One month later, 0.5 mg. of rattlesnake venom per kilogram of body weight was injected intravenously. The roentgenogram shows the vascular beds one week later, following an injection of bismuth.

kidney, however, the large vessels were well preserved, and though the finer vessels showed a poor injection of bismuth they were demonstrated much better than in the left kidney.

Microscopically, the right kidney displayed marked swelling and congestion of the glomeruli, marked swelling of the interstitial blood vessels and a few interstitial hemorrhages. The endothelium of the arterioles was swollen. The tubular epithelium was swollen and granular.

The glomeruli of the left kidney were even more markedly swollen, almost uniformly obliterating Bowman's space. The same endothelial swelling of the arterioles was seen here as on the right, with the same vascular congestion. However, swelling of the tubular epithelium was much more marked, especially in the convoluted tubules, where the lumen was almost obliterated.

## CHILLING

A group of dogs was subjected to low temperatures for varying periods of time; bismuth was injected into the renal vascular bed, and subsequently roentgenograms were made to determine the changes in the blood vessels. The animals were shaved over the trunk, anesthetized and packed in ice for the desired length of time.

*Dog 16.*—A normal dog was chilled for one hour. Bismuth was injected into the kidneys, and roentgenograms demonstrated a wide vascular bed with the arteria recti extending almost to the outer margin of the cortex.

*Dog 17.*—Dog 17 was treated exactly the same as dog 16. During the time of chill lasting one hour, no urine was secreted from either kidney. At the end of this period, bismuth was injected into the kidneys, and a normal bed was demonstrated.

*Dog 18* (fig. 10).—The dog was denervated on June 9, 1931. Two weeks later, the animal was chilled for ten minutes, and bismuth was injected into the kidneys. The cortical vascular bed of the left (denervated) kidney showed extensive injection, while that of the right was but poorly delineated. The left kidney weighed 38.5 Gm., while the right weighed 29.5 Gm.

Microscopically, the capillaries of the glomeruli and the interstitial vessels contained little blood or bismuth, and the tubular epithelium was pale and shrunken in the right kidney, whereas in the left kidney the vessels were all distended with blood, and the tubular epithelium was swollen and deeply stained.

*Dog 19.*—The left kidney was denervated on Oct. 29, 1930. On November 16, the region of the kidneys was shaved and ice applied locally for twenty minutes. Bismuth was then injected. The vascular bed of the left kidney showed somewhat better injection than that of the right kidney. The difference, however, was not marked.

On microscopic section, the glomeruli of the right kidney were of moderate size, and their capillaries contained a moderate number of red cells. The tubular epithelium appeared normal; the interstitial blood vessels contained a moderate amount of blood. In the left kidney, the glomeruli were larger, their capillaries contained much more blood, and granules of bismuth were found to have penetrated many of them. The interstitial vessels contained more blood than those in the right kidney; the tubular epithelium was rather well preserved.

*Dog 20.*—The left kidney was denervated on June 28, 1930. On July 23, an ice pack was applied for one hour, during which the right kidney secreted 10.5 cc. and the left 9 cc. of urine with a specific gravity of 1.040 and 1.038 and an ammonia nitrogen content of 31 and 29 mg. per hundred cubic centimeters, respectively. Injection at this time showed a more widely open vascular bed in the left kidney, with considerably better injection into the finer vessels.

Microscopically, the glomerular capillaries in the right kidney were rather markedly dilated and contained little blood, although the granules of bismuth had penetrated into many of them. The blood vessels and tubules were essentially normal. In the left kidney, the glomeruli were distended with blood, and here again granules of bismuth had penetrated to the capillary loops. The interstitial vessels contained a considerable amount of blood, and the tubular epithelium was well preserved.

*Dog 21.*—The left kidney was denervated on Sept. 30, 1930, and the injection of bismuth was made on November 5. The animal was chilled for one and one-

half hours. The roentgenogram showed marked obliteration of the vascular bed in each kidney, more marked in the left.

Microscopic section of the right kidney displayed moderate congestion of glomerular capillaries and interstitial blood vessels. The tubular epithelium was moderately swollen, pale and vacuolated. The section of the left kidney displayed normal glomeruli and about the same degree of tubular degeneration as was seen

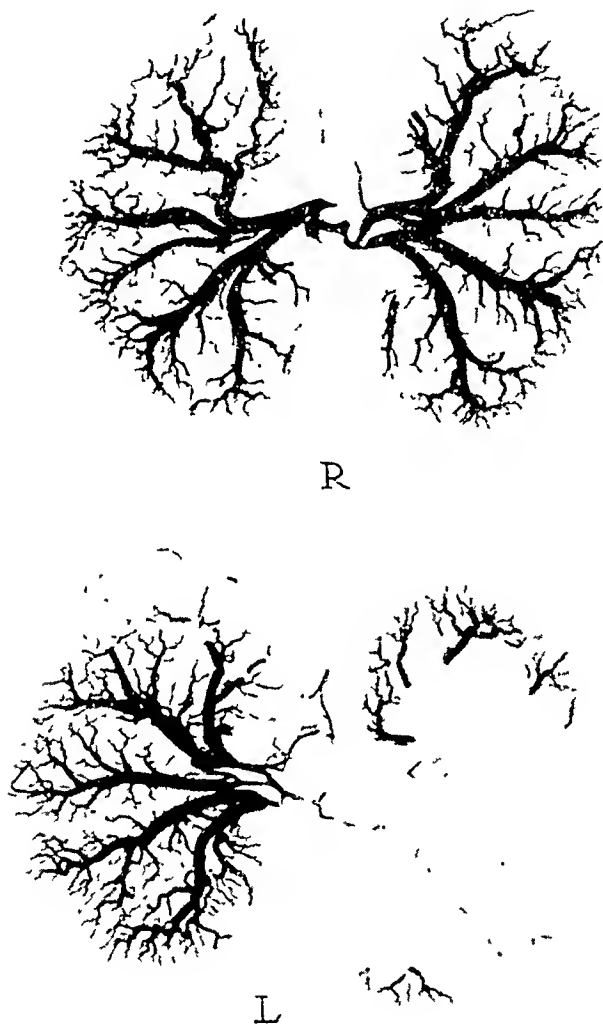


Fig. 10 (dog 18).—The left kidney was denervated on June 9, 1931. Two weeks later, ice was applied over the kidneys for ten minutes, and then bismuth was injected into the renal vascular beds. One branch of the left renal artery was occluded during the injection, so that a poor injection was obtained in the corresponding half of the kidney.

in the right kidney. The injected bismuth had penetrated the finer vessels, and fragments were seen in the glomerular tufts.

After a short period of chilling, it appeared that the vascular bed of the normal kidney was constricted as a result of a spasm of the

small vessels, while the vascular bed of the denervated kidney showed little change. With a longer period of chilling, however, the result was variable, and in many cases the vascular beds of both kidneys were dilated.

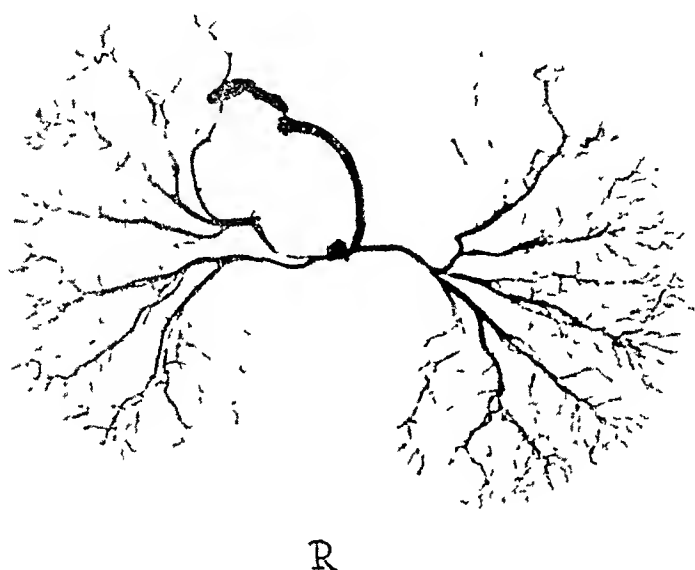


Fig 11 (dog 22).—The left kidney was denervated on Oct. 9, 1930. Two weeks later, daily chilling was begun and continued for eleven weeks. Then bismuth was injected into the renal vascular beds.

#### REPEATED CHILLING

A group of animals were subjected to daily chilling for a period of from two to six months. The animals were placed in an ice chest after

having been shaved and daily subjected to a temperature of about 0 C. for a period gradually increasing from five minutes to an hour.

*Dog 22* (fig. 11).—The left kidney was denervated on Oct. 9, 1930. Daily chilling was begun on October 20. Bismuth was injected on Jan. 7, 1931. The kidneys were found to be equal in size and showed good injection. The left kidney showed a cortical vascular bed of much more marked extent than the right.

Histologic sections of the right kidney showed the glomeruli to be distended, the capillary loops filled with blood and containing granules of injected bismuth. In a few, the endothelium of the afferent vessels was swollen, and occasionally hemorrhage into Bowman's space was seen. The arterioles displayed some swelling of their endothelium. The tubular epithelium was swollen and granular, and considerable granular and hyaline debris was present in the lumina. In the left kidney, the glomeruli presented a normal appearance and contained many fragments of the bismuth. Bowman's space was everywhere free from contents. The tubular epithelium was everywhere well preserved.

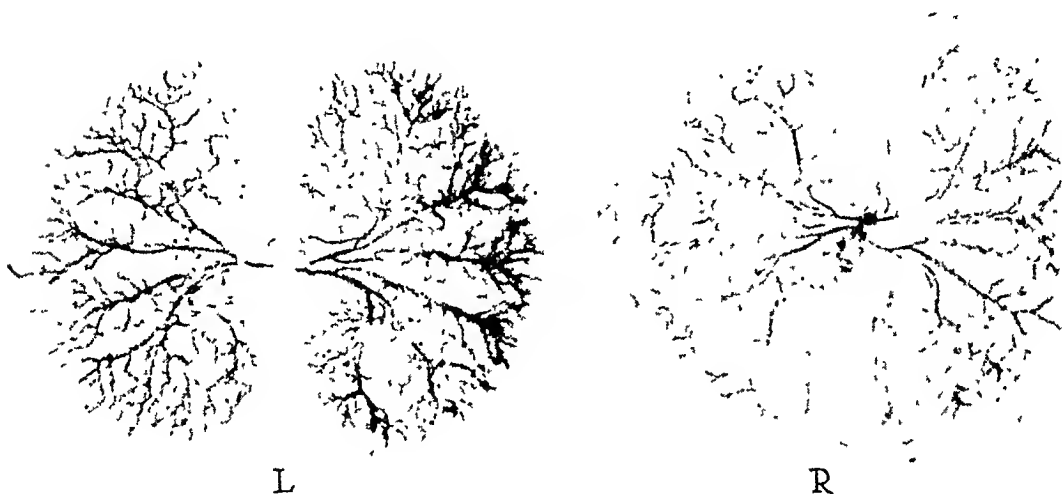


Fig. 12 (dog 23).—The left kidney was denervated on June 11, 1930. One week later, daily chilling was begun and continued for three months. Then bismuth was injected into the renal vascular beds.

*Dog 23* (fig. 12).—The left kidney was denervated on June 11, 1930. Daily chilling was begun on June 18, and on September 12 the urine was collected separately from both kidneys, and bismuth was then injected into the kidneys. The vascular bed of the left kidney was normal, while that of the right kidney was almost completely obliterated. The left kidney weighed 33.5 Gm and the right kidney, 27 Gm.

Microscopically, the histologic section of the right kidney demonstrated marked dilatation and congestion of the glomerular capillaries, as well as of the interstitial blood vessels. The tubular epithelium was swollen and granular in some areas, and in others it was shrunken and broken down. Considerable granular debris was present in the tubular lumina. The sections of the left kidney showed evidence that the congestion was much less marked than in the right, not only in the glomeruli, but also in the interstitial vessels. The tubular epithelium was somewhat better preserved than that in the right kidney.

*Dog 24.*—The left kidney was denervated on June 11, 1930. The daily chilling was begun on June 18, and bismuth was injected into the kidneys on July 21. The vascular bed of the left kidney was found to be practically normal. In the right kidney there was practically no injection of bismuth into the finer vessels, and there was slight beading of the larger vessels.

*Dog 25.*—The left kidney was denervated on June 11. Daily chilling was begun on June 18. On September 9, the dog died. The cause of death was pneumonia. The roentgenogram of the vascular beds of the kidneys showed evidences of marked injury. The left, however, was far better preserved than that of the right kidney. There was some evidence of beading in the large blood vessels of both kidneys.

In the microscopic section of the right kidney, the glomeruli were slightly congested. The endothelium of the afferent vessels was swollen, and occasionally granular debris or erythrocytes were seen in Bowman's space; little bismuth had reached the glomeruli. The tubular epithelium was well preserved. The section of the left kidney showed that the congestion of the glomeruli was more marked. The tubular epithelium was swollen and more granular; otherwise, the picture was the same as in the right kidney.

*Dog 26.*—The left kidney was denervated on Sept. 24, 1930. Daily chilling was begun on October 20, and bismuth was injected into the kidneys on March 30, 1931. There was practically no difference in the vascular beds on the two sides nor was there any marked change. Microscopically, the histologic section of the right kidney showed a marked congestion and dilatation of the glomerular capillaries, with slight swelling of the vascular endothelium in both the capillaries and the arterioles, and marked cloudy swelling of the tubular epithelium. In the left kidney, the picture was essentially the same, except that evidence of congestion was not as marked, and endothelial changes in the blood vessels were not seen.

#### COMMENT

By means of these roentgenologic studies of the vascular bed of the kidney we have demonstrated a definite dilatation of the vascular bed following denervation, which, persisting for at least two months, is in agreement with the microscopic findings of de Gironcoli. In an old animal having renal lesions best classified as chronic nephritis, we have demonstrated an increase in the size of the organ, as well as in its vascularity, following denervation.

To determine the efficacy of the denervation, epinephrine was used; in the normal dog, it was found to cause an obliteration of the renal vascular bed, but in the unilaterally denervated animal, the denervated kidney was found to be practically unaffected. This is of importance in that it demonstrated the effectiveness of the denervation that we had achieved.

In a small group of animals to which mercuric bichloride was administered, no change in the vascular bed was demonstrable. Repeated doses of uranium similarly showed no effect. Further work with these metallic poisons is now under way.

In the response to the injection of a vascular poison, such as rattlesnake venom, the normal and denervated kidneys showed marked differences so far as the toxic effect was much more marked on the denervated kidney. This was apparent in the obliteration of the cortical vascular tree in the roentgenogram, as well as in the microscopic observations. We deal here with the probability that the response of the normal kidney involves a prompt vasoconstriction. The denervated kidney, unable to protect itself, receives the bulk of the toxic material and is more severely injured. Cortical edema then results, and this in turn leads to the obliteration of the lumen of the smaller blood vessels of the cortex, while corresponding vessels of the intact kidney retain their normal size.

That this same relation seems to be operative with infectious processes might be evident from an examination of normal and denervated kidneys in animals that have died from distemper (dogs 6517, 6124 and 6038). In these cases, too, the denervated kidney shows greater change than the normal kidney.

In examination of the roentgenograms of these kidneys, we would stress particularly the importance of the degree of injection shown by the cortical vessels rather than the changes that are observable in the larger vessels of the kidneys. Localized constrictions or beadings similar to those demonstrated by Graham<sup>16</sup> in human arteriosclerotic kidneys were observed to occur spontaneously in the kidneys of old dogs, as well as following injections of rattlesnake venom and after repeated chilling. In the old animals, they are probably due to focal sclerotic change in the vessels. Following the injection of rattlesnake venom and repeated chilling, they are ascribable to the swelling of the intima seen microscopically and possibly to localized areas of vasomotor spasm.

Of special importance to us were the results obtained by repeated chilling of animals daily over a period of months, because we presumably deal here with the result of a purely autonomic effect.

Maintenance of the functional normality of individual organs or of an organism as a whole involves constant autonomic vascular adjustment. While this is accepted as axiomatic in the consideration of physiologic problems, it is not infrequently overlooked in investigations in the pathologic field, where we are apt to concentrate our attention on a presumptive exogenous pathogenic factor rather than on the failure of organic adjustment.

This is true even for resistance to infection. The herpes virus affords a striking example. Constantly present about the labial and

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16. Graham, R. S.: A Study of the Circulation in the Normal and Pathological Kidney with Roentgenographic Visualization of the Arterial Tree Including the Glomeruli, *Am. J. Path.* 4:17, 1928.



nasal orifices in certain persons, it gives rise to no pathologic lesion except at certain times (chilling, menstrual cycle, gastro-intestinal upset) when the vasomotor control of the skin is altered.<sup>17</sup>

This being true for the skin with its relatively small blood supply and low metabolic demand,<sup>17</sup> how much more important must be the proper autonomic coordination in organs of great metabolic demand such as the kidney?

We have been able to show that bacteria (*B. coli*), the intravenous injection of which is normally followed by the appearance of albumin, red blood corpuscles and casts (as well as of the bacteria) in the urine (and which therefore must be considered as exogenous toxic agents) do not bring about this pathologic effect if the autonomic innervation to the kidney (vasomotor control) is severed.<sup>18</sup> We have also shown that the chill associated with bacterial invasion is associated with vasoconstriction in the kidney, and we believe the assumption to be justified that the initiation of deranged function of the kidney under these conditions is predicated on two factors—autonomic dysfunction (relative ischemia of the kidney) *together with* an exogenous (and possibly an endogenous) toxic factor.

The effect of chilling offers a number of interesting problems. When the animal chills as the result of bacterial invasion, a peculiar muscular tremor occurs to which has been ascribed the increase in temperature that usually follows in its wake. As a matter of fact, we have demonstrated<sup>19</sup> that the muscular phenomenon is merely part of the autonomic reaction during which the peripheral vascular bed is contracted and the splanchnic bed (liver, spleen, gastro-intestinal tract) is dilated. It is, of course, associated with the entrance of adrenal secretion in large quantities into the circulation.

When the chill is induced by external cold, a similar effect takes place in the splanchnoperipheral balance. But when the chill is severe and prolonged, this autonomic balance is disturbed, and even the vascular bed of the kidney becomes dilated. The roentgenograms of the kidneys of animals chilled for short periods of time or following local applications of ice to the peripheral tissues show a distinct difference in the effect on the denervated, as compared with the normal, kidney, the denervated kidney being relatively vascular. With prolonged and fatal exposure, this difference is no longer apparent.

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17. Petersen, W. F., and Levinson, S. A.: The Skin Reaction, Blood Chemistry and Physical Status of Normal Men and Clinical Patients, *Arch. Path.* **9**:151, 1930.

18. Milles, G.; Müller, E. F., and Petersen, W. F.: Studies in Renal Denervation, *Proc. Soc. Exper. Biol. & Med.* **28**:351, 354 and 561, 1931.

19. Müller, E. F., and Petersen, W. F.: Ueber das Verhalten der Skelett-Muskulatur in Schüttelfrost, *München. med. Wchnschr.* **74**:1218 and 1276, 1927.

These experiments would indicate that in the normal kidney a vasoconstrictor effect occurs during the time of a short chilling and make it probable that the urinary changes that are found under such conditions (albumin, red blood cells, casts, etc.) are in part the result of temporary ischemia. In an autonomically labile person, they would naturally be more pronounced than in a normal person.

We have the impression, too, that the association of chilling with the onset of acute nephritis is of pathogenic importance. Under such conditions, the renal tissue, with its relative ischemia, is apparently more readily damaged than when the vascular supply is adequate.

The constriction associated with an acute chill in the onset of nephritis may be the factor determining whether or not the damage produced by an infectious agent will be sufficiently great to become permanent or whether it will be merely transient.

In the consideration of the causative factors that are involved in the complicated pictures of chronic nephritis, hyperpiesis and the more acute forms that are found in the arteriolosclerotic types, autonomic dysfunction with repeated arteriolar spasm has been suggested. Jaffe,<sup>20</sup> for instance, presented histologic evidence that makes very probable a relationship of repeated arteriolar spasm of the afferent arterioles of the glomerulus to the pathologic picture of the arteriolosclerotic kidney. Kylin<sup>21</sup> discussed the presumptive instability of the autonomic status of such patients, as well as characteristic peculiarities of their blood chemistry.

So, too, the "wear and tear of modern life" must involve greater demands on the autonomic apparatus and produce autonomic maladjustment leading directly or indirectly to vasomotor dysfunction in susceptible organs.

Repeated chilling producing such a spasm, when involving blood vessels of the kidney, may finally bring about anatomic changes. In our experiments in which dogs were chilled daily over long periods of time, a distinct difference was found rather uniformly and regularly involving particularly the finer vessels, those in the normal kidney being practically obliterated, while those in the denervated kidney remained unaffected.

In view of these pronounced changes that have resulted from such repeated spasms of the renal vessels it seems logical to assume that these must play some rôle in the production of certain types of nephritis.

The interpretation of the results of a histologic examination of the kidneys of dogs always offers certain difficulties. It has been recog-

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20. Jaffe, R. H.: Vascular Changes of Kidney in Hypertension, *Am. J. M. Sc.* **169**:88, 1925.

21. Kylin, E.: *Die Hypertoniekrankheiten*, Berlin, Julius Springer, 1926.

nized that fat, for instance, is almost always a normal constituent in the tubular epithelium and other apparent, degenerative changes in the tubular epithelium occur so constantly as to be without significance unless extremely marked. On the other hand, it is unusual to observe definite changes in the glomeruli or in Bowman's capsule. In this series, we observe normally moderate vascular dilatation following denervation, such as was seen by de Gironcoli, a variation in the penetration of the injected material into the finer blood vessels and particularly into the glomerular capillaries. This penetration of injected material was only roughly estimated, but usually it was found to be more marked in the denervated than in the normal kidneys. Eppinger<sup>22</sup> observed a marked decrease in the penetration of india ink following injury with mercuric chloride and took this as an indication of a diminution in the blood flow. Following the injection of snake venom, marked congestion and some interstitial extravasation of blood and red cells are seen in the tubules, especially in the denervated kidneys. After repeated daily chilling, marked congestion and some endothelial swelling of the arterioles and capillaries are found in normal kidneys as compared with the usual picture seen in denervated kidneys.

#### CONCLUSIONS

As shown by roentgenologic study of the vascular bed of the kidney in dogs, renal denervation is followed by a long persisting dilatation of the cortical blood vessels.

Chilling of the animal for a short period of time causes a definite vasoconstriction of the intact kidney as compared with the denervated organ; with prolonged and fatal chilling, this difference is no longer apparent. Daily chilling over long periods of time results in marked change of the vascular bed of the normal kidney, while the denervated kidney remains unaltered. A relation of the chill to the onset of acute nephritis, as well as of the effect of repeated spasm of the renal vessels (as with repeated chilling) to the pathogenesis of nephritic changes is made probable.

A vascular poison, such as snake venom, causes greater injury to the denervated kidney, the normal kidney presumably protecting itself through vasoconstriction. The effect appears to be true for infectious diseases as well (distemper).

No change in the vascular bed was observed with single doses of mercuric bichloride or with repeated doses of uranium acetate.

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22. Eppinger, H.; Laslo, D.; Rein, F., and Schurmeyer, A.: Circulatory Changes in the Pathological Kidney, *Klin. Wchnschr.* 9:633, 1930.

# EXPERIMENTAL INFARCTION OF THE GLOMERULI IN DOGS

## II. BLOOD PRESSURE IN CHRONIC RENAL INSUFFICIENCY \*

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In former years, the clinical and anatomic characteristics of those forms of Bright's disease that are associated with arterial hypertension were interpreted on the postulate that the changes in the kidneys were primary, and that, consequently, all clinical and anatomic alterations resulted from decrease in renal function. Gradually, however, a different concept has developed which conceives Bright's disease as a clinical and anatomic complex in which the renal damage is only part of a general disease such as occurs in arteriosclerosis, glomerulonephritis or essential arterial hypertension.

Crucial facts that would aid in evaluating these two divergent concepts should come from patients in whom death occurs from disease that affects only the kidneys, or from animals in which the kidneys alone are altered by experimental procedures. Chronic forms of renal insufficiency in which anatomic changes are localized to the kidneys are rare in human beings and, as a result, the usual conception of Bright's disease is derived from patients in whom many organs are simultaneously altered. Likewise, many of the experimental methods for studying Bright's disease can be objected to because the noxious substances used, even though they change the kidneys preponderantly, may affect many other tissues.

The majority of instances of human Bright's disease are combinations, clinically, of renal insufficiency, edema, arteriosclerosis, arterial hypertension and uremia. The pathologist, particularly, is often confronted with anatomic inconsistencies in associating these alterations causally with a proportional decrease in renal tissue. He observes cases in which hydronephrotic atrophy induces death, and he may not find hypertrophy of the left ventricle, arteriosclerosis or edema. On

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\* From the Pathological Laboratory of the Presbyterian Hospital and the Norman Bridge Pathological Laboratory of Rush Medical College of the University of Chicago.

the other hand, he observes cases in which renal damage is not severe, yet arteriosclerosis, hypertrophy of the left ventricle and edema may be marked. It is on account of many of these inconsistencies that additional doubt has been introduced against the concept that renal damage alone explains all of the clinical and anatomic alterations observed in acute and chronic Bright's disease.

#### METHOD USED IN PRODUCING RENAL INSUFFICIENCY

Miller and Apfelbach<sup>1</sup> described a method of producing chronic renal insufficiency that alters only renal tissue, thereby avoiding some of the criticism advanced against experimental methods in which tissues other than the kidneys might also be damaged. By their method, infarction of glomeruli follows the injection of a suspension of charcoal particles into the renal arteries, the particles being of such size that they occlude the lumina of the glomerular capillaries. This method of inducing chronic renal insufficiency was chosen because of the following factors:

1. The interference with renal function is a direct result of the experimental procedure, and no other changes in the body result except the healing of the surgical incisions through the wall of the body in the lumbar regions.

2. The renal insufficiency is comparable to that which occurs in human beings, namely, retention of nitrogenous end-products in the blood plasma, fixation of the specific gravity of the urine and excretion of albumin and casts in the urine.

3. The renal insufficiency is chronic. Some dogs have lived for several months.

4. It has been suggested by some experimenters that, in order to secure an elevation of blood pressure in experimental Bright's disease, the reduction of kidney tissue must be accomplished in such a manner that the destroyed tissue is left in situ. The method described by Miller and Apfelbach meets this objection to the excision method of producing renal insufficiency, for the retrogressive changes that result in the glomeruli and convoluted tubules subsequent to infarction resemble the changes observed in human chronic Bright's disease.

The authors concluded that there is a constant syndrome characteristic of chronic renal insufficiency in dogs induced by gradual reduction of renal tissue. Also, in the course of this later study on blood pressure in relation to renal insufficiency, they have become even more impressed with the constancy of the clinical and anatomic alterations that are associated with what might be called "pure renal insufficiency." The following characteristics are always present and occur in direct proportion to the degree of reduction of renal tissue: (1) low and fixed

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1. Miller, E. M., and Apfelbach, C. W.: *Arch. Path.* 4:193, 1927.

specific gravity of the urine, (2) loss of flexibility in the capacity of the kidneys to vary the rate of excretion, (3) polyuria, (4) albuminuria, (5) casts, (6) retention of nitrogenous end-products in the blood, (7) decrease in the carbon dioxide-combining power of the plasma and (8) decrease in body weight.

On the other hand, some of the characteristics of human Bright's disease were never observed. Edema did not occur. Anemia was never severe and usually did not exist. Fatty change of the kidneys, so frequently found in subacute and chronic glomerulonephritis, was not encountered. Convulsions, muscular twitchings and stupor were not observed until the carbon dioxide-combining power of the blood reached a value of from 15 to 25 per cent.

#### MANOMETER USED IN STUDY OF BLOOD PRESSURE IN DOGS WITH RENAL INSUFFICIENCY

The relationship of arterial hypertension to experimental chronic renal insufficiency had not been definitely settled in the report by Miller and Apfelbach. They stated:

We have tried to record the blood pressure by bands adapted to the thigh, but the results were so inconsistent in normal animals that no confidence is placed in them. Alterations in the weight of the heart are also difficult to interpret. We have attempted to arrive at a standard for the heart and body weight, but this also is too variable. The weight of the heart is apparently more dependent on the previous activity of the dog than on body weight. The variations in thickness of the papillary muscles and trabeculae carneae are also not usable.

For the purpose of studying the blood pressure in dogs with renal insufficiency, we<sup>2</sup> designed a mercury manometer that could be used daily without anesthesia. A 22 gage needle in the femoral artery distal to Poupart's ligament connects the blood stream with a mercury manometer. Only a mean systolic pressure can be obtained, but this mean systolic pressure is only a few millimeters lower than the mean systolic pressure obtained simultaneously in the femoral artery through a large glass cannula. The error in this method is a constant one, whereas our experience with cuff methods was such that no constancy of determinations could be obtained. For the purpose, therefore, of recording permanent alterations in the mean systolic blood pressure, we believe that this method is a reliable one.

#### THE PROBLEM OF THE RELATIONSHIP OF EXPERIMENTAL RENAL INSUFFICIENCY TO BLOOD PRESSURE AS RECORDED IN THE LITERATURE

The conclusions arrived at by experimenters concerning the relationship of arterial hypertension to renal insufficiency are as inconsistent

2. Jensen, C. R., and Apfelbach, C. W.: *Arch. Path.* 6:99, 1928.

as are those entertained by clinicians. Passler and Heincke<sup>3</sup> studied the blood pressure after removing portions of renal tissue in dogs. Ether anesthesia was used, and a cannula was inserted into the femoral artery and attached to a mercury manometer. When 50 per cent of the kidney had been removed, they found polyuria, an elevation of blood pressure of from 15 to 29 mm. of mercury, and hypertrophy of the wall of the left cardiac ventricle in 29 per cent of their animals.

Janeway,<sup>4</sup> using dogs, excised one kidney and tied off branches of the renal artery of the other kidney. Some of the dogs lived from thirty-nine to one hundred and sixty-three days. A Riva-Rocci cuff was used in making the blood pressure observations. An elevation of blood pressure in several animals was recorded, the highest being 37 mm. of mercury.

Backmann,<sup>5</sup> using a Trendelenburg tonometer, found no changes in blood pressure after bilateral nephrectomy, but after removal of one kidney and a small piece of the other, he noted a rise of 18 mm. of mercury.

Anderson,<sup>6</sup> using rabbits, made determinations of blood pressure from the central artery of the ear. All of one kidney and part of the other were removed, about 70 per cent in all. The animals lived from one hundred to two hundred days after the operation. Two of the rabbits apparently died from renal insufficiency; others had a moderate degree of renal insufficiency, and some were recovering. None showed a rise of blood pressure.

Cash,<sup>7</sup> in a series of ten dogs, using several different operative procedures, came to the following conclusions: 1. A rise of systolic and diastolic pressures in most instances and a rise of diastolic pressure in all is obtained when a reduction of at least 50 per cent of the renal substance is effected, provided a portion of renal tissue that has been deprived of its circulation is allowed to remain in situ. 2. Either removal of one kidney alone or infarction of one kidney alone is without effect on the blood pressure. 3. The increase in blood pressure reaches its height within a few days following operation, after which it tends to return to normal. Cash made his observations on blood pressure by means of a Kolls metal cuff together with a mercury manometer and a sphygmograph, and he continued to record them for from two to four months. Routine observations were also made on the nonprotein nitrogen and total chloride contents of the blood, with occasional determina-

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3. Passler and Heincke: *Verhandl. d. deutsch. path. Gesellsch.* **9**:99, 1905.

4. Janeway, T. C.: *Proc. Soc. Exper. Biol. & Med.* **6**:108, 1908-1909; *Am. J. M. Sc.* **145**:625, 1913.

5. Backmann, E. L.: *Ztschr. f. d. ges. exper. Med.* **4**:63, 1914.

6. Anderson, H. C.: *J. Exper. Med.* **39**:707, 1924.

7. Cash, J. R.: *Bull. Johns Hopkins Hosp.* **35**:168, 1924.

tions of the blood creatinine, and on phenolsulphonphthalein excretion. It seems, though, that no marked degree of renal insufficiency was produced in any of his dogs.

A method of obtaining what might be fairly classified as pure renal insufficiency was reported by Hartmann, Bolliger and Doub.<sup>8</sup> They irradiated the kidneys of dogs in several ways. Acute and chronic changes were produced, including tubular degeneration, sclerosis of vessels and, finally, shrunken, sclerotic kidneys. Undoubted renal insufficiency was produced in those that survived, as measured by retention of blood metabolites, lowered carbon dioxide-combining power of the plasma and diminished phenolsulphonphthalein excretion. The authors stated: “. . . in the final stages systolic blood pressure reached 230 and the diastolic 150 in some instances.”

Thus, it may be seen that experimental methods so far have not been in agreement as to the causal relationship between renal insufficiency and arterial hypertension.

#### OBSERVATIONS ON NATURAL ELEVATIONS OF BLOOD PRESSURE IN DOGS

During the course of our study of the blood pressure in dogs, we observed that there were fluctuations in the mean systolic pressure that seemed to be concomitant with changes in the temperature of the room. In order to confirm this observation, a room was constructed in which the temperature could be automatically regulated.<sup>9</sup> A continuously recording Tycos thermometer was installed that recorded the dry bulb and wet bulb temperatures. The carbon dioxide content of the room did not fluctuate appreciably. The observations were made on large dogs that had become accustomed to manipulation. The diet consisted of dried beef myocardium, corn meal, milk, sawdust and, on every other day, fresh meat. Ample water was supplied. The records were made immediately before the dogs were fed. Also, changes in temperature were induced in the room after determining the blood pressure.

In charts 1 and 2 are recorded the continuous dry bulb and wet bulb temperatures of the room, the barometric pressure and the blood pressure as obtained by the mercury manometer described by us. The contents of these charts indicate that there is a marked change in the systolic blood pressure that occurs as a result of sudden changes in the room temperature. There may be other modifying factors that make this relationship more or less intense, such as the barometric pressure

8. Hartmann, F. W.; Bolliger, A., and Doub, H. P.: *Am. J. M. Sc.* **172**: 487, 1926.

9. A fund was obtained from the Board of Managers of the Presbyterian Hospital to construct this constant temperature room.



and humidity, but it seems that the most important factor is the temperature outside of the body. We believe that fluctuation of blood pressure in dogs of from 20 to 30 mm. of mercury are meaningless in relationship to renal arterial hypertension unless the climatic conditions under which the dogs live are controlled.

A further objection to some of the experimental evidence advanced in regard to the production of arterial hypertension in dogs is the state-

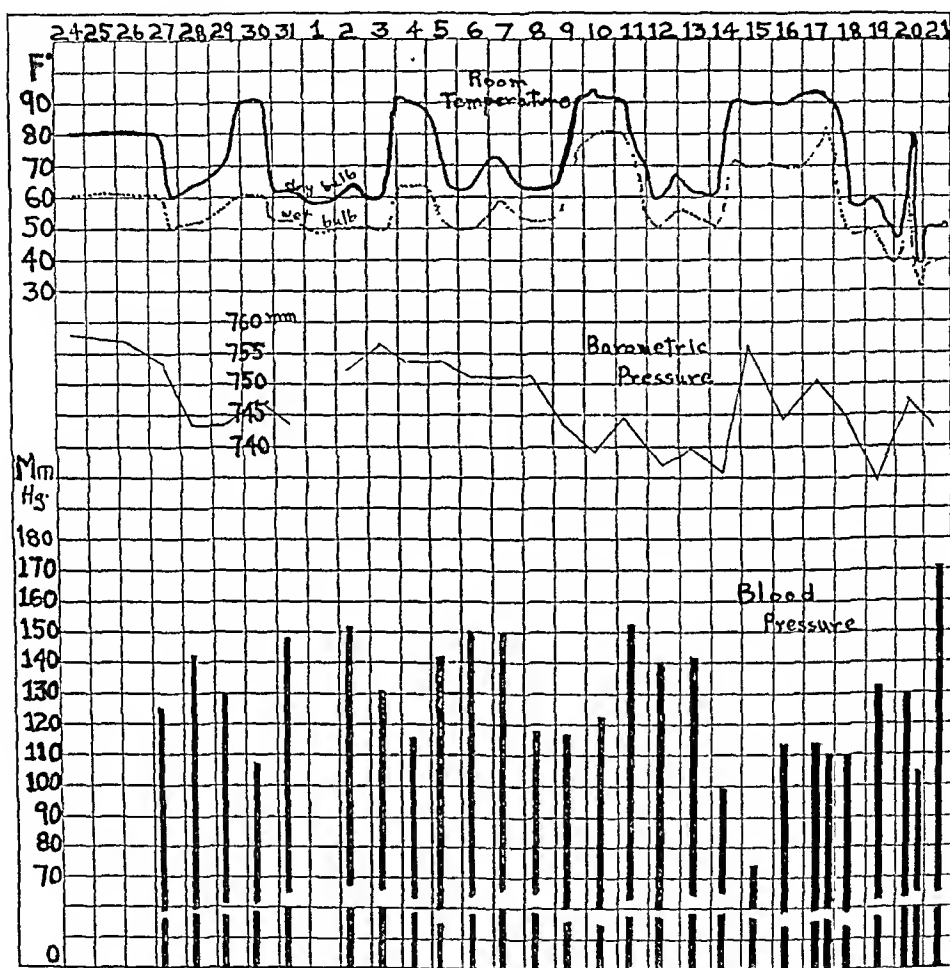


Chart 1.—Alterations in blood pressure due to changes in room temperature. The chart records daily observations that were made in the case of a female dog the average weight of which was 50 pounds (22.7 Kg.). The dog had been in the laboratory for a few months and was accustomed to the necessary manipulations. The two upper transverse lines record the continuous dry bulb and wet bulb temperatures as recorded on a Tycos thermometer. The lower transverse line represents the barometric pressure, which was recorded once a day. The broken vertical lines indicate the blood pressure as determined by the mercury manometer described by us.

ment that the blood pressure of dogs is normally as high as 200 mm. of mercury. We have never observed a dog with a natural arterial

hypertension. It is true that during the first few days of observation, while the dog is frightened and resistant, the systolic pressure is sometimes as high as 175 mm. of mercury, but after the animal becomes accustomed to having observations made, the blood pressure is found to be constant, regardless of weight, sex or age, provided the outside temperature remains fairly constant.

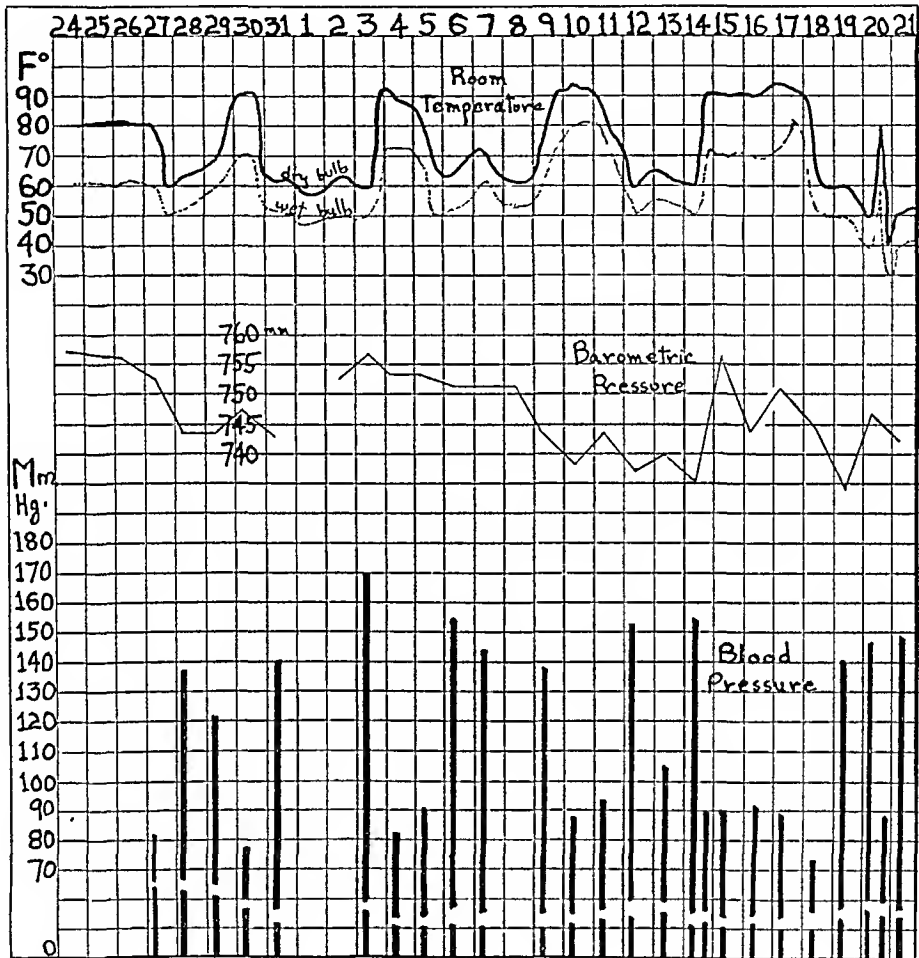


Chart 2.—Alteration in blood pressure due to changes in room temperature. The chart records daily observations that were made in the case of a male dog the average weight of which was 45 pounds (20.4 Kg.). This dog had been in the laboratory for several months and had been used for no experimental work except as a control for the living conditions under which the dogs with renal insufficiency were kept. For further explanation of the chart, see the legend for chart 1.

Still another factor that may be confusing is that a dog must be placed under observation almost daily. If long periods ensue between manipulations, the animal may again, as at first, suffer from fright, and an elevation of systolic pressure may result.

OBSERVATIONS ON BLOOD PRESSURE IN DOGS WITH EXPERIMENTAL  
RENAL INSUFFICIENCY

During a period of three years, in which seventy-seven female dogs were studied, no rise of systolic blood pressure was observed as a result of renal insufficiency. From this group of seventy-seven animals, we

TABLE 1.—*Protocol of a Dog with Pure Renal Insufficiency*

The results recorded in this table were obtained during the period extending from Aug. 17, 1926, to Nov. 17, 1927. The dog was a female mongrel about 2 years old. The determinations of blood pressure began late in the course of the renal insufficiency, because the apparatus had not yet been devised. As we have found that the blood pressure of dogs averages from 120 to 130 mm. of mercury, we believe it is not assuming too much if that average is adopted for the probable preoperative blood pressure of this dog

Date	Weight, Lb. (Kg.)	Specific Gravity of Urine	Phenol- sulphon- phthalein Excretion, per Cent per Hour	Urea Nitrogen, Mg. per 100 Cc. of Blood	Nonprotein Nitrogen, Mg. per 100 Cc. of Blood	Carbon Dioxide- Combining Power of Blood Plasma, per Cent by Volume	Blood Pressure, Mm. of Mercury	Hemo- globin, Gm. per 100 Cc. of Blood
8/17/26	33 (15)	1.018	....	20.2	38.4	51	...	15.6
8/17/26	Charcoal injected into both kidneys through the renal arteries							
8/19/26	.....	1.006	....	26.6	40.4	..	...	....
8/23/26	30 (13.6)	1.010	....	70.9	102.6	..	...	....
8/26/26	.....	.....	.....	.....	.....	..	...	15.0
9/ 9/26	27 (12.22)	1.018	61.0	22.4	43.0	46	...	....
10/ 7/26	Charcoal reinjected into right renal artery							
10/12/26	24 (10.9)	1.009	....	74.0	96.4	..	...	....
10/16/26	.....	1.010	....	125.8	156.2	..	...	....
11/16/26	22 (10)	1.008	14.0	133.3	198.1	34	...	13.5
11/22/26	.....	1.010	....	83.3	100.5	..	...	....
12/15/26	21 (9.5)	1.008	....	142.5	160.0	..	...	....
12/23/26	.....	1.008	....	149.0	241.5	..	...	....
1/ 3/27	.....	1.006	18.0	126.5	165.5	30	...	....
1/17/27	22 (10)	1.008	....	69.9	91.5	..	...	....
1/19/27	.....	.....	....	114.0	171.8	..	...	....
2/ 7/27	.....	1.008	....	63.5	86.5	..	...	....
2/21/27	.....	.....	....	84.5	105.0	..	...	....
2/26/27	.....	1.006	....	64.5	102.2	..	...	....
3/ 7/27	.....	1.008	....	104.2	118.5	..	...	....
3/17/27	.....	1.008	....	107.5	171.1	..	...	....
4/ 4/27	.....	.....	....	102.0	168.3	31	...	12.5
4/11/27	27 (12.22)	1.010	....	55.5	80.9	41	...	....
5/ 4/27	.....	1.008	21.0	62.5	78.5	33	...	....
6/14/27	.....	.....	....	88.0	101.9	..	110	....
6/27/27	26 (11.8)	1.008	18.0	67.8	96.0	26	118	11.0
7/ 7/27	28 (12.7)	1.006	....	108.1	158.6	28	124	....
7/14/27	.....	1.013	....	76.2	106.0	38	120	....
7/25/27	.....	1.012	26.0	30.8	62.5	..	122	....
7/27/27	Charcoal reinjected into left kidney							
8/19/27	.....	1.015	....	61.5	115.5	31	112	....
9/15/27	30 (13.6)	1.012	21.0	63.5	126.6	..	118	....
10/ 7/27	.....	1.016	18.0	71.4	126.0	..	126	12.0
10/18/27	.....	1.010	....	88.9	153.2	28	110	....
10/24/27	26 (11.8)	1.007	....	61.5	107.5	30	108	....
11/ 7/27	22 (10)	1.006	15.0	80.0	138.0	..	110	....
11/10/27	.....	1.005	....	80.0	170.8	24	118	....
11/17/27	24 (10.9)	1.007	....	117.6	221.6	21	114	....

At this time, the dog was inadvertently given 5 Gm. of ammonium chloride by a technician and as a result died in two days with a drop of the carbon dioxide-combining power to 16 per cent.

No edema occurred at any time. The arterial system was free from arteriosclerosis, and the heart was not enlarged. The kidneys together weighed 40 Gm. They resembled the usual contracted form described and illustrated by Miller and Apfelbach.<sup>1</sup>

were able to secure only ten that lived long enough to be regarded as presenting chronic renal insufficiency. Infection and excessive renal insufficiency interfered with the use of the others and curtailed the experiments to an acute stage.

TABLE 2.—*Protocol of a Dog with Pure Renal Insufficiency*

The results recorded in this table were obtained during the period extending from Jan. 5, 1929, to Aug. 19, 1929. The dog was a short-haired black and tan female terrier and weighed 24 pounds (10.9 Kg.)

Date	Weight, Lb. (Kg.)	Specific Gravity of Urine	Phenol- sulphon- phthalein Excretion, per Cent per Hour	Urea Nitrogen, Mg. per 100 Cc. of Blood	Nonprotein Nitrogen, Mg. per 100 Cc. of Blood	Carbon Dioxide- Combining Power of Blood Plasma, per Cent by Volume	Blood Pressure, Mm. of Mercury	Hemo- globin, Gm. per 100 Cc. of Blood
1/ 5/29	24 (10.9)	1.042	64	16.0	28.0	54	138	15.6
1/14/29	.....	1.038	..	.....	.....	..	130	....
1/16/29	.....	.....	..	.....	.....	..	124	....
1/29/29	25 (11.3)	1.048	58	12.6	24.8	50	122	15.2
2/12/29	Right kidney removed							
2/24/29	.....	1.028	..	16.8	30.2	48	126	....
3/ 4/29	22 (10)	1.030	40	22.8	41.6	51	118	14.9
3/27/29	.....	1.032	..	36.4	61.2	36.2	114	15.8
4/ 2/29	One branch of left renal artery ligated							
4/ 3/29	21 (9.5)	1.018	..	.....	.....	....	116	14.8
4/ 5/29	.....	.....	..	12.5	31.8	50.4	...	15.7
4/19/29	Charecoal injected into left kidney through renal artery							
5/ 8/29	19 (8.6)	1.024	27	80.0	106.0	27.7	118	15.2
5/28/29	.....	1.016	..	70.0	134.0	24.9	122	14.7
6/ 3/29	20 (9)	1.004	32	83.4	130.9	30.5	114	13.5
6/10/29	.....	1.006	..	96.0	169.5	25.8	110	12.1
6/17/29	18 (8.2)	1.008	..	115.0	160.2	30.5	104	11.9
7/16/29	.....	1.002	..	135.0	245.5	27.9	90	11.4
8/ 2/29	17 (7.7)	1.004	28	147.0	248.0	24.9	116	12.4
8/ 4/29	.....	.....	..	.....	.....	....	130	....
8/19/29	18 (8.2)	1.006	21	135.1	203.8	22.3	104	16.0
8/19/29	(Repeat)	.....	..	134.0	217.7	16.4	108	....

At this time the dog had become very weak, barely able to walk and slightly stuporous, and it refused to eat or drink. Consequently, the animal was killed by air embolism in order that the tissues might be secured in a fresh state. There was no gross or microscopic evidence of arteriosclerosis. The heart was not enlarged. There was no edema of the extremities or of the serous cavities. The left kidney weighed 15 Gm. The right, of course, was absent. The microscopic changes were those described by Miller and Apfelbach.<sup>1</sup>

The animals were observed for from several days to a few weeks before renal insufficiency was induced. During this period, the systolic blood pressure was determined, and the animal was not operated on until a constant level was reached. The phenolsulphonphthalein excretion was determined, the microscopic characteristics, the specific gravity, the ammonia, total nitrogen and chloride concentrations of the urine were observed, and the level of the nitrogenous end-products in the blood and

the carbon dioxide-combining power of the blood plasma were obtained. In tables 1 to 3, we have recorded the protocols of three dogs. The results listed are those necessary to indicate the degree of renal insufficiency and the usual blood pressure levels in such animals.

TABLE 3.—*Protocol of a Dog with Pure Renal Insufficiency*

The results recorded in this table were obtained during the period extending from July 26, 1929, to Dec. 2, 1929. The dog was a female poodle, weighing 16 pounds (8.6 Kg.)

Date	Weight, Lb. (Kg.)	Specific Gravity of Urine	Phenol- sulphon- phthalein Excretion, per Cent per Hour	Urea Nitrogen, Mg. per 100 Cc. of Blood	Nonprotein Nitrogen, Mg. per 100 Cc. of Blood	Carbon Dioxide- Combining Power of Blood Plasma, per Cent by Volume	Blood Pressure, Mm. of Mercury	Hemo- globin, Gm. per 100 Cc. of Blood
7/26/29	16 (7.3)	1.042	45	23.1	49.1	41	128	17.1
7/27/29	.....	1.048	..	25.2	44.9	43	116	16.0
7/29/29	.....	1.046	..	....	....	..	128	....
8/ 2/29	.....	....	..	....	....	..	136	....
8/ 3/29	15 (6.8)	1.040	..	....	....	..	128	....
8/ 4/29	.....	....	..	22.4	41.6	47	122	....
8/ 7/29	Charcoal injected into the right renal artery							
8/11/29	.....	1.038	..	26.0	42.0	41	128	....
8/16/29	Charcoal injected into the left renal artery							
8/26/29	.....	1.024	..	37.0	51.8	49	80	....
9/ 5/29	.....	1.020	32	51.0	96.5	34	104	12.5
9/ 7/29	16 (7.3)	1.018	..	....	....	..	116	....
9/14/29	.....	....	..	80.6	102.9	40	130	12.0
9/19/29	.....	1.022	..	73.0	92.0	32	116	12.2
9/24/29	....	....	..	....	....	..	118	....
10/ 2/29	.....	1.026	28	76.8	92.0	38	128	16.0
10/ 8/29	.....	1.030	..	....	....	..	124	....
10/11/29	16 (7.3)	....	..	....	....	..	120	....
10/16/29	.....	1.034	..	....	....	..	144	....
10/24/29	.....	1.030	..	64.4	91.4	44	128	14.7
10/31/29	14 (6.4)	....	..	59.0	95.0	39	136	....
11/ 3/29	.....	....	..	....	....	..	142	....
11/ 9/29	.....	1.025	..	....	....	..	128	....
11/13/29	.....	....	..	....	....	..	124	....
11/21/29	.....	1.024	..	24.4	40.8	51	136	15.7
11/25/29	.....	....	..	....	....	..	140	....
11/26/29	Charcoal reinjected into right kidney							
11/29/29	.....	1.018	..	116.8	234.0	38	116	....
12/ 2/29	.....	1.020	..	384.0	500.0	27	110	(Dead)

The right kidney was the site of an acute purulent nephritis as a result of the injection of charcoal. The left kidney weighed only 14 Gm. No enlargement of the heart, edema or gross histologic changes of arteriosclerosis were present.

#### SUMMARY AND CONCLUSIONS

Glomerulonephritis and nephrosclerosis are interpreted at present as general vascular diseases rather than as primary renal diseases with subsequent vascular changes. Also, only part of the clinical manifestations of these two diseases are interpreted as the result of renal insufficiency.

Pure renal insufficiency can be produced in dogs by injecting charcoal particles into the renal arteries, thereby causing infarcts in the glomeruli. The changes that result in animals with pure renal insufficiency of this kind are retention of nitrogen in the blood, decrease in the ability of the kidney to concentrate and dilute urine, decrease in the carbon dioxide-combining power of the blood plasma and decrease in the specific gravity of the urine. Edema and arterial hypertension do not occur.

In the medical literature there are discrepancies between the results of experimenters who have attempted to study arterial hypertension in relationship to renal insufficiency. Some of this discrepancy is due to the failure to take into account the changes in blood pressure that result from alterations in climatic conditions, and some error has occurred because the instruments used in determining blood pressure have given erroneous results.

With changes in room temperature of from 30 to 40 degrees F., a dog's blood pressure may vary as much as 80 mm. of mercury, the pressure going up in a cold room and dropping when the room becomes warm. In addition, the blood pressure of dogs varies easily from fright, and consequently repeated determinations must be made in order to keep the animals accustomed to the procedure.

When these conditions that alter the blood pressure of dogs are taken into consideration, arterial hypertension does not occur in the presence of marked renal insufficiency.

# General Review

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## SYPHILITIC MYOCARDITIS \*

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CHICAGO

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At the present time syphilitic myocarditis is frequently discussed. While some authors believe that it is often encountered in syphilis in its later stages, others believe that it is rare. Not only does this disagreement prevail at the bedside, but it is also carried to the autopsy table, and even histologic study of the myocardium leads to different interpretations.

My purpose in this paper is, first, to attempt to give a critical review of the literature of syphilitic myocarditis and to relate whatever facts justify this diagnosis anatomically; second, to report results of a study of the myocardium in cases that showed syphilitic aortitis with involvement of the aortic valve. This paper is confined to myocardial changes in acquired syphilis. It deals with myocardial changes called variously chronic syphilitic myocarditis, fibrous form of syphilitic myocarditis, latent syphilitic myocarditis and active syphilitic myocarditis. For convenience, the term syphilitic myocarditis will be used in this paper and will refer only to the lesions just mentioned. Under this term, gummas of the heart, gummatous myocarditis and changes of the heart due to hereditary syphilis are not included, as these conditions are considered to be different from the lesions included in the term syphilitic myocarditis. The gummatous lesions and changes due to hereditary syphilis will be mentioned only when necessary to give a more complete review and understanding of the main subject.

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## CHRONOLOGICAL REVIEW OF THE LITERATURE

The first case described in the literature which might possibly have been a case of syphilitic myocarditis was reported by Lancisius in the year 1718. He mentioned an aneurysm of the heart in a patient who had received treatment with mercury. He discussed briefly a possible relationship of the aneurysm to the treatment with mercury. He gave, of course, no detailed studies of the myocardium. But this case is referred to by later writers as possibly a case of syphilitic myocarditis.

Ricord in 1845 is credited with having been the first to describe gumma of the heart. He also described a hemorrhagic infiltration of the myocardium extending over the entire cardiac wall. But because of the facts that the endocardium and the pericardium in this portion were thickened and that the endocardium, in addition, was covered by a thrombus, it is likely that the so-called hemorrhagic infiltration of the myocardium was an infarcted area rather than evidence of myocarditis.

Lebert, who shortly afterward described another case of gumma of the myocardium, made no reference to a possible myocarditis.

Wilks presented before the Pathological Society of London, in 1856, a case of fibroid growth in the ventricular septum of the heart. This has subsequently been referred to as the first case of syphilitic myocarditis. Wilks stated that the growth in the septum looked like a gumma, but he was not convinced that the lesion was syphilitic in origin. He further mentioned that he had seen similar tumors in the muscles and in the tongue and viscera of those who died of inveterate syphilis. In a report on this heart, Baly and Bristowe stated that histologically fibroid nuclei and tuberculous material were found. The muscle fibers were more or less changed. There was fibroid tissue with exceedingly delicate, interlacing fibrils and a meshwork of fibers. Baly and Bristowe remarked, however, that there is no reason to suppose that this case differed in any important particular from other cases that have been brought to the notice of the society as examples of fibroid degeneration of the heart.

Virchow, in 1858, in his paper on the nature of the constitutional syphilitic diseases, mentioned a case (case VII) of gummas of the heart. He pointed out that the myocardium was replaced in patches by a soft, richly vascularized and edematous tissue, found in close vicinity to the gumma. Microscopically, many round cells were found, partially arranged in rows with newly formed connective tissue fibers and, in some places, fatty degenerated cells. Some of the muscle fibers were distinctly atrophic. Virchow raised the question whether or not syphilitic myocarditis exists unaccompanied by the formation of gummas. He concluded that such an occurrence is possible, but neither stated on what basis the diagnosis could be made nor mentioned a case that he himself had seen.



Dittrich in 1852 mentioned a myocarditis (case XI) with an abundance of fibrous tissue in a patient with scars in the liver and spleen and syphilitic scars in the cranium. However, he did not conclude that the myocarditis was syphilitic.

A few years later Virchow, in his "Die krankhaften Geschwuelste" (1864-1865), said that up to a few years ago involvement of the heart in syphilis was regarded as belonging in the realm of fairy tales, but that in the course of the last few years a series of cases of gummas of the heart had been reported. He further stated that, in addition to gummatous myocarditis, fibrous myocarditis occurs in syphilis, but that it is difficult to prove that such cases are syphilitic. He said that in examining the hearts in a large number of cases of syphilis one finds many with multiple fibrous scars in the myocardium, without any demonstrable cause but syphilis. He recommended further research in syphilitic myocarditis.

E. Wagner in 1866 reported a case of syphilitic myocarditis in a stillborn child. His diagnosis was based mainly on the fact that the mother of the child showed signs of secondary syphilis. Histologic examination revealed an increase in connective tissue between the muscle fibers and some fatty degeneration of muscle fibers.

Lanceraux in 1866 differentiated strictly between interstitial syphilitic myocarditis and gummatous myocarditis. Both types of myocarditis are often combined. Grossly, there are white and yellowish areas of connective tissue throughout the heart. Histologically, many cellular elements are present, with a richly vascularized connective tissue.

Aufrecht in 1866 and Mueller in 1868 described the cases of syphilis of the heart in which autopsies were performed at the Pathological Institute of Berlin during the years from 1863 to 1868. Aufrecht mentioned 4 cases of fibrous myocarditis, 1 of which also showed gummatous myocarditis. Mueller recorded 4 cases of interstitial fibrous myocarditis. He stated that the myocardial lesions were syphilitic in origin: first, because no other etiologic factor could be demonstrated to explain the lesions; second, because such types of myocarditis often coincide with gummatous myocarditis, and, third, because there was a similarity between the changes in the myocardium and those found in syphilitic orchitis and syphilitic interstitial hepatitis. Neither author gave histologic details.

Fowler in 1868 described a case of sudden death of a man 45 years old. The heart was enlarged; the lower third of it was occupied by a yellowish-white, semitranslucent material of firm consistency. This material was found in the endocardium and, in some portions, extended through the entire ventricular wall. Histologically, this new material was essentially a fibronucleated structure, having a close resemblance to the ordinary early stage of fibroid degeneration of syphilis. There

was a new formation of connective tissue that seemed to have started from the walls of blood vessels. The coronary arteries, however, were not mentioned.

Morgan in 1872 reported 4 cases, in 3 of which autopsies were performed. One was that of a child, a few months old, who showed gummas in the liver and white spots in the heart. The latter were taken as evidence of syphilis with no other justification but the coincidental finding of gummas in the liver.

Hertz in 1873 described a case of aneurysm of the aorta and syphilitic pneumonia. In the heart a moderate amount of connective tissue between the muscle fibers was seen with lymphocytes and spindle-shaped cells. The belief was expressed that these observations might lead to the conclusion that the patient had had syphilis of the heart.

Baeumler in 1874 stated that in syphilis the heart might show gummas or fibrous myocarditis, sometimes fatty changes and, according to Lanceraux, amyloid degeneration.

Jullien in 1879 stated that he had collected 19 cases of gummas and myocarditis. Gummas and fibrosis of the myocardium sometimes occurred simultaneously. He ventured to say that the occurrence of isolated syphilitic fibrosis of the myocardium is rare, and that if it occurs it is the result of primary diffuse myocarditis. No details were given.

Erhlich in 1880 stated that syphilitic disease of the heart is rare. He described a case of fibrous myocarditis with small grayish-white, necrotic foci, well demarcated, some of which were surrounded by hemorrhagic zones. Histologically, the myocardium showed marked cellular infiltration, many capillaries and some pigment. The muscle fibers close to the necrotic foci were partly atrophic. Many fibers showed no nuclear staining. There was a more or less prominent invasion by pus cells. The necrotic foci were interpreted as areas of coagulation necrosis brought about by endarteritis syphilitica obliterans of the smaller blood vessels. The author believed that such primary changes in the blood vessels may cause secondary syphilitic myocarditis.

Teissier in 1882 reported a case of sudden death in a woman 27 years old. A few vegetations were found on the aortic valve, with fibrous myocarditis and gummas in the myocardium. Histologically, there were diffuse cellular infiltration and perivascular infiltration by round cells; the intima of some of the blood vessels was thickened. The muscle fibers were atrophic.

Chvostek in the same year mentioned a case of fibrous syphilitic myocarditis in the left ventricle, the ventricle being dilated. The walls of the papillary muscles were thickened and, in several places, interspersed with white, dense connective tissue fibers. The myocardium was firm and very pale and had a peculiar fatty shine. Chvostek offered no microscopic description.

Bramwell in 1884 pointed out that in syphilis the arteries adjacent to, or in the midst of, fibroid patches in the heart might be narrowed by endarteritis obliterans.

Paul in 1884 stated that syphilitic lesions may appear in two forms: either as ordinary sclerotic myocarditis accompanied by other syphilitic lesions or as gummy tumors.

Green in 1887 reported a case of aneurysm of the heart with yellowish-white, gummatous-looking material. The posterior coronary artery or a branch of it was involved in this disease and apparently obliterated, but the remainder of the coronary arteries appeared healthy. A clear clinical history of syphilis was specifically mentioned as proof of the syphilitic nature of these changes.

Orth in 1887 stated that the combination of gummas in the heart and fibrous inflammation is commonly encountered. He believed that primary syphilitic fibrosis exists, even though he admitted that it might be secondary to syphilitic endarteritis.

Ashby in 1887 reported a case of sudden death. The heart showed white patches of fibrosis. Microscopically, there were round cells, spindle cells and connective tissue fibers. Some of the arteries were thickened in places. The case was diagnosed as possibly syphilitic myocarditis.

Ziegler in 1887 stated that gummas of the heart are rare; that fibrous myocarditis is found somewhat more frequently, as a result of hereditary or acquired syphilis, and that some of the cases reported in the literature as cases of syphilitic fibrous myocarditis are more likely cases of arteriosclerosis.

Bargum in 1888 reported the case of a 45 year old man. The left ventricle was somewhat hypertrophic; the coronary vessels were normal. In the wall of the left ventricle several fibrous spots were noted, which were stellate. Microscopically, the fibrous spots showed much connective tissue, a few cells and many blood vessels. The arteries were the seat of perivascularitis. The muscle fibers were atrophic. Small nodular areas of round cells with necrosis also were demonstrable. The author believed that this was a case of gummas of the myocardium combined with syphilitic myocarditis.

Mauriac in 1889 stated that the heart is the rarest location of syphilis. From 25 to 30 such cases were known. He stated that if gummas are absent, it is difficult to differentiate syphilitic sclerosis of the heart from other types of sclerosis. The vascular changes found in such cases consist mainly of periarteritis, with the predominating changes in the adventitia. The intima usually is also infiltrated, and the lumen, in some instances, is completely obliterated.

Buchwald in 1889 remarked that syphilitic myocarditis is a frequent finding. He held that the changes are characteristic pathologico-

anatomically. The characteristic features, however, were not mentioned. He concluded that a large number of persons afflicted with syphilis sooner or later show syphilitic myocarditis with or without diseased blood vessels.

T. Lang, who in 1889 had observed 44 cases of syphilis of the heart, stated that syphilitic myocarditis always is fibrous or gummatous. In his "Vorlesungen ueber Pathologie und Therapie der venerischen Erkrankungen," he further stated that the diagnosis of syphilis in cases of chronic myocarditis must be supported by the anamnesis and the presence of other manifestations of syphilis. He did not give histologic details.

Schwalbe in 1890 mentioned that syphilitic interstitial myocarditis is a disease per se and occurs independent of the presence of gummas or infarcts. He did not refer, however, to his own observations.

Saccharyin in 1890 stated that the basis for the diagnosis of syphilis of the heart lies in the history of the case and the simultaneous finding of syphilis in other internal organs.

Bogossowsky in 1891 mentioned 2 cases of syphilitic myocarditis in which the diagnosis of syphilis was based on the observation that the condition of the patients improved after the administration of potassium iodide.

Cohnheim, in 1891, in describing a case of gumma in the intra-ventricular septum, did not mention syphilitic myocarditis. Juergens in 1891 mentioned a case of cardiac gumma among 4 cases of so-called primary tumors of the heart, but made no reference to myocarditis.

Stockton in 1891 stated that in a patient who had syphilis, and who was intolerant to mercury, endocarditis and nephritis had developed. He did not mention the myocardium nor give a detailed description of the heart.

Mracek in 1892 stated that syphilis of the heart is rare. He divided the changes of the heart brought about by syphilis into two groups. To the first group he assigned the direct products of syphilis, namely, the gumma and a specific inflammation which, according to its end-stage, is called fibrous sclerosing myocarditis, and to the second, changes occurring secondarily to lesions of the first group, i. e., atrophy of muscle fibers, regressive changes brought about by vascular lesions, fibrosis and aneurysms. The histologic criteria of syphilitic myocarditis are old fibrous tissue in addition to newly formed connective tissue and granulation tissue. The portions of the myocardium that are situated close to the branches of the coronary vessels or close to the vasa vasorum are involved first. Among the 3 cases which the author reported was a case showing fibrous myocarditis with vascular changes and necrosis.

Palma in 1892 described a case of so-called syphilitic myocarditis. A portion of the descending branch of the left coronary artery was obliterated. The myocardium showed granulation tissue also in portions

that were supplied by branches of the coronary artery that were not completely obliterated. There was an abundance of connective tissue, but no calcification. This case also showed gummatous orchitis. Sections of the coronary revealed nothing indicative of syphilis. It seems unwarranted to speak of syphilis of the coronary artery or of the myocardium in this case merely because a gummatous orchitis was found coincidentally.

Rolleston in 1893 found in the heart of a 34 year old man who died suddenly many pea-sized nodules that consisted histologically of round cell infiltrations. Weight is put on the fact that old syphilitic changes were found in the testis.

Mracek in 1893 reviewed the literature on syphilis of the heart. He had collected from the literature reports of 9 cases of fibrous myocarditis, 8 of fibrous and gummatous myocarditis and 15 of myocarditis combined with pericarditis or endocarditis. In addition, he reported a case of chronic fibrous myocarditis with obliterating endarteritis and necrosis of the myocardium. He emphasized the fact that the diagnosis of syphilis can be made only through analogous syphilitic changes in other organs.

Pearse in 1893 described clinical observations in a case of angina pectoris. No postmortem observations were given.

Kockel in 1893 reported 2 cases. The first showed fibrous myocarditis with partial aneurysm of the heart and a small gumma. Histologically there was an increase in connective tissue which was poor in nuclei, but which showed in a few places a small, circumscribed cellular infiltration. Several newly formed capillaries were also noted. The smaller arteries showed inconstant intimal thickenings, in not a few instances so advanced as to lead to marked narrowing of their lumina. He ventured the opinion that the myocarditis was a syphilitic inflammation, the result of an obliterating endarteritis. The second case occurred in a man, aged 19, who showed a circumscribed nodule on the intima of the descending branch of the right coronary artery, which was thrombosed in this region. The heart showed many white spots. The nodule consisted histologically of accumulations of triangular and spindle-shaped nuclei, with a few round cells. There was some necrosis in the center. Syphilis as the causative agent seemed probable, because of the youth of the patient and because of the fact that only one nodule was found. There was no other manifestation of syphilis, however. There is no detailed histologic description of the heart.

Barlaro in 1893 reported 2 cases of syphilis of the heart, both of which showed angina pectoris clinically. In one case a gumma of the heart was combined with fibrous myocarditis.

Birsch-Hirschfeld in 1894 attributed a diffuse, chronic myocarditis, among other agents, to syphilis. He classified syphilis of the myo-

cardium into gumma and diffuse myocarditis. Gummatous foci may lead to the formation of an aneurysm and rupture of the heart.

Hektoen in 1894 reported the case of a 6 weeks old child. The myocardium showed many whitish, round areas, measuring up to 1 cm. in diameter, which were of a homogeneous surface and which showed no calcification. Histologically, there was an infiltration of round and oval nucleated cells around smaller vessels. The larger subepicardial vessels were not involved. There was no proliferation of endothelial cells. The adventitia contained more connective tissue fibers. The interstitial changes were so marked as to lead to the gross diagnosis of gummas.

Loomis in 1895 classified the syphilitic lesions of the heart into four groups, namely, gummas, fibroid induration, amyloidosis and endarteritis obliterans, often producing infarcts. He stated that, besides the formation of gummatous tumors, syphilis gives rise to an indurated myocarditis, a lesion that in its later stage is hardly distinguishable from fibroid disease due to other causes. It is possible to arrive at the origin of these new growths only by inference from the history of the patient, and from the presence of constitutional syphilis, especially gummatous tumors in other situations. It seems probable, as suggested by Ziegler, that the inflammatory induration as a consequence of congenital or acquired syphilis is much more common than the development of gummas. Loomis assumed that a syphilitic fibrous induration presents localized, well defined fibroid areas situated in the substances of the heart, or diffuse irregular patches of new fibroid tissue with endarteritis obliterans of minute arteries or interstitial myocarditis due to a partial absorption of gummas and replacement by connective tissue. He had seen 15 cases of fibroid myocarditis in which the diagnosis was made by microscopic examination, 3 of which were, beyond all doubt, of syphilitic origin.

Philips in 1896 reported that among 4,000 cases in which autopsies were performed at the Pathological Institute of Kiel, 99 were cases of syphilis and 397 were possibly cases of syphilis. None of these 496 cases showed a syphilitic fibrous process in the heart.

Stolper in 1896 found, among statistical material consisting of 2,995 cases, 61 of acquired syphilis. One case showed a gumma in the heart and another a possible diffuse gummatous infiltration of the heart. He presented the following questions which had not been answered: Does syphilis of the heart produce fibrous productive inflammation, or is the fibrous tissue found in such hearts scar tissue replacing old necrotic areas? What relations are there between the disease of the muscles and the vascular changes? Are the changes caused by syphilis or by something else? Syphilis might have been the cause in 2 of his cases. The myocardium showed a cellular infiltration of the adventitia of the

smaller vessels and some endarteritis obliterans. The author preferred to speak of fibrous myocarditis in syphilitic persons instead of syphilitic myocarditis.

Grassmann in 1897 remarked that the prevalent opinion of the rarity of syphilis should be corrected, and that only gummas of the heart are rare. His paper is purely clinical, but gives a historical review of this subject.

Philipps in 1897 mentioned fibrous myocardial changes in syphilitic patients. He spoke of 25 cases in which there were gummas, syphilitic infiltration or fibrosis, but he gave no histologic data.

Rosenbach in 1897 mentioned among syphilitic conditions of the myocardium, gumma, fibrous inflammation, endarteritis obliterans with infarcts and new formation of connective tissue, but stated that none of these lesions shows enough characteristics to allow a diagnosis of syphilis.

Herrick in 1897 stated that fibrous and gummatous forms of syphilitic myocarditis had been observed. The fibrous form is characterized by grayish areas or streaks in the myocardium, single or multiple. Areas when fully fibrous differ in no respect from fibrous myocardial patches due to coronary obstruction with consequent myomalacia and subsequent fibrous patches. The concomitant gumma is unmistakable evidence of syphilis. Complete exclusion of coronary disease also reveals the syphilitic nature of this form of myocarditis. It is to be remembered that coronary endarteritis obliterans syphilitica may be a cause of myomalacia and fibrous change. Care must be taken lest one too frequently assign a syphilitic origin to fibrous myocarditis, especially because of the fact that Orth stated that fibrous myocarditis is clearly recognized as syphilitic only when gummas are also present.

Adler in 1898 demonstrated 2 cases of Addison's disease brought about by gummas of the suprarenal glands. One of these cases revealed an interstitial myocarditis. There was a mere cellular infiltration with some coagulation necrosis. No endarteritis or periarteritis was demonstrable. The heart in the second case also showed evidence of periarteritis and endarteritis of the coronary arteries, as well as some hemorrhage.

LeCount in 1898 reported gummas in the heart in a case of congenital syphilis. There were white areas, circular in form, in the myocardium. Histologically, a marked infiltration by round or fibrillated cells was found most marked around the arterioles. Multiple foci of interstitial myocarditis were present, with polymorphonuclear leukocytes and also areas of degeneration and necrosis. The title "gummata," the author stated, is a matter of preference.

E. Lang in 1899 remarked that there is a simple fibrous syphilitic myocarditis with multiple fibrous cicatrices in the cardiac muscle. In

such cases partial aneurysms have been repeatedly observed. He did not give the histologic features by means of which such a myocarditis might be recognized.

Rosenthal in 1900 pointed out that the first state of syphilitic myocarditis is a specific endarteritis, while the end-stage is a fibrous scar. He differentiated a gummatous form and an interstitial or fibrous form. In the description of a clinical case of syphilitic myocarditis, he reached the conclusion that in syphilis myocarditis may develop on the basis of functional overactivity of the heart.

Grassmann in 1900 and 1901, in papers dealing with clinical aspects, reached the conclusion that in early syphilis the heart is frequently involved.

Stevens in 1901 stated that syphilis of the myocardium manifests itself as a more or less fibroid induration, as a gummatous growth or, rarely, as amyloid degeneration. Fibroid induration or interstitial myocarditis is usually secondary to syphilitic endarteritis. But the myocarditis also can result from the direct action of the specific poison on the muscle cells or on their sheaths, since a few cases have been noted in which no lesion of the coronary arteries could be detected.

Krehl in 1901 pointed out that syphilis of the heart is not rare. All parts of the heart may be involved. Syphilitic myocarditis may be the result of vascular changes, may be present in the form of gummas or may manifest itself as a diffuse interstitial inflammation.

Wagner and Qwiatkowski in 1903 reported a case of syphilis of the heart. Grossly, dry and yellowish-gray areas were found in the myocardium. Histologically, spindle-shaped cells and necrotic portions were demonstrated. Evidence of endarteritis was shown. There was much fibrous tissue in the midst of the muscle tissue.

Quensel in 1903 stated that the fibrous myocarditis found in the heart in cases of syphilis offers nothing characteristic of syphilis, either grossly or histologically. A diagnosis of syphilitic myocarditis is a diagnosis based on probability only. In a study of 121 cases of syphilis he found fibrous myocarditis eight times in patients who were older than 40 years, and five times in patients younger than 40. But he was not certain that syphilis was the underlying cause of the myocarditis.

Busse in 1903 reported a case of syphilitic inflammation of the heart and of the external muscles of the eye. The heart showed a recent inflammation and scar tissue. Many endothelial cells and giant cells were present. There was an intimal proliferation of the smaller arteries. The muscle fibers were the seat of a fatty metamorphosis, but no necrosis was demonstrable. A search for tubercle bacilli gave negative results. In the author's opinion the peculiar mixture of scar tissue and



recent inflammation combined with the presence of giant cells is characteristic of syphilis. It is probable that this case presented gummatous myocarditis.

Cowan in 1903 showed a picture (fig. 11) of the heart in a case of syphilis in the secondary stage. The lumina of the arteries were but little interfered with. There was newly formed connective tissue, which was much vascularized and which extended between the individual muscle fibers. He stated that some scars of the myocardium may be the result of gummas.

Romanow in 1904 reported a case of productive myocarditis which was thought to be syphilitic in origin. He stated that syphilis of the heart is not too rare.

Stockmann in 1904 reviewed 76 reports of cases which he had collected from the literature and reported 4 cases which he had observed. Many of these cases showed myocardial fibrosis in addition to gummas.

Adler in 1904 stated that primary interstitial myocarditis may occur very early in syphilis. There is cellular infiltration in the adventitia of the small arterioles. From these proliferations as starting points, strands of cellular infiltration transverse the adjacent myocardium. The muscle fibers, which first are normal, degenerate gradually, while others become atrophic. The media of the arteries become fibrous, the intima proliferates and an obliteration of the arterioles results. An extreme fibrous degeneration of the cardiac muscle takes place. Cardiac syphilis is not rare; on the contrary, it is rather a common disease.

Renvers in 1904 found among 2,000 cases of cardiac disease 26 which were anatomically cases of syphilis of the heart, 3 of which showed gummas. He did not give a histologic description of these cases.

Huchard in 1905 stated (page 885) that paralysis of the heart may be caused by chronic syphilitic myocarditis. He did not give any histologic details.

Takeya in 1906 described a case of gummatous pericarditis and a case of gummas in the myocardium. But he did not mention syphilitic myocarditis.

Stoeltzner in 1906 reported a case of syphilitic myocarditis in a child 2 years old. There were white and yellow spots in the myocardium, which were connected with one another. Histologically, young granulation tissue was found. No giant cells or necrotic portions could be demonstrated. The patient had had lobar pneumonia six weeks previously. This case, even though reported as a case of syphilitic myocarditis, showed nothing characteristic of syphilis.

Buschke and Fischer in 1906 found in a case of congenital syphilis in a child 3 weeks old an interstitial diffuse myocarditis. There was

a marked increase of cellular connective tissue, which was edematous. A few circumscribed infiltrations with central necrosis and destruction of muscle fibers were noted. Infiltrations by lymphocytes were found surrounding smaller branches of the coronary arteries. Many spirochetes could be demonstrated by the use of the Levaditi method. They were found around the infiltrated vessels and within their lumina.

Janeway and Waite in 1907 reported a case of gummas of the heart and liver. There were many eosinophils in the myocardium in addition to the gummas.

Herxheimer in 1907 pointed out that an infiltration by round cells along the blood vessels of the interstitial tissue is characteristic of syphilitic fibrous myocarditis. The muscle fibers suffer secondarily by pressure and as a result of vascular disturbances. Scar tissue is found mainly in the left ventricle, close to the apex. Gummatous myocarditis is often combined with fibrous myocarditis.

Pitzner in 1908 reported 7 cases of syphilis in which fibrous myocarditis was found. The author believed that the localization of the fibrosis in the heart, namely, in the region of the interventricular septum, is characteristic of syphilis. He also mentioned that in these cases sclerosis of the coronary vessels was often missing. Thorel, however, in discussing the changes, stated the belief that all of them could be explained on a nonsyphilitic basis.

Landois in 1908 reported 3 cases of syphilis of the heart. The first case was that of a 6 year old girl afflicted with congenital syphilis. The heart showed several gummas. In the second case, which occurred in a 46 year old man, the heart showed white scars throughout the myocardium. Histologically, connective tissue was present in these areas, with only a few nuclei. Cells were found surrounding small vessels, the lumina of which were obliterated. The author believed that the diagnosis of syphilis of the heart was justified because the larger branches of the coronaries showed no arteriosclerotic changes, while the small branches were partly obliterated. (It might be stated that the patient had died of diphtheritic enteritis.) In the third case, which occurred in a 56 year old woman, the heart was the seat of many gray, scarlike foci. The intima of the coronary arteries was smooth. Histologically, many round cells and scar tissue were found between the muscle fibers. The author stated that the inflammatory reaction as such is not characteristic of syphilis. He mentioned, however, that there is no other disease known which produces such an outspoken fresh interstitial inflammation involving the entire cardiac muscle and later appears only in certain foci.

Adami and Nicholls in 1909 stated that syphilis is an infrequent cardiac disease. It may lead to gummatous foci, to fibroid induration, to amyloid infiltration and to endarteritis obliterans, often causing

infarction. The indurative inflammation is perhaps the most common. The authors did not state the histologic criteria of these syphilitic conditions.

Romberg in 1909 remarked that, more frequently than gumma, diffuse interstitial myocarditis is found in the heart in cases of syphilis.

Powell in 1909 stated that syphilitic myocarditis invariably occurs either immediately adjacent to gummas or secondary to, and in the territory of, specific arteritis. Syphilis may affect the myocardium in the form of syphilitic arteritis with secondary necrosis and ultimate fibrosis, in the form of gummas or in the form of a diffuse chronic myocarditis of specific nature affecting a considerable portion of the heart. It is, however, doubtful whether this form does not originate in the fusion of scattered gummatous depositions. He further stated that knowledge of syphilitic myocarditis is mainly derived from post-mortem observations in cases in which by no means all of the patients died with cardiac symptoms.

Berblinger in 1910 described a case of what he called gummatous myocarditis. Histologically, many round cells were found, partly diffuse, partly localized. There were many fibroblasts present, but no well defined nodules consisting of endothelial cells. There was a marked vascularization of the granulation tissue. Some sections showed many connective tissue fibers which were rich in nuclei, while others revealed old fibrous connective tissue without nuclei. A few fields showed perivascular infiltration. There was no caseation.

Warthin in 1911 maintained that a special form of myocarditis exists which is due to the presence of spirochetes resulting from congenital syphilis. In cases of this condition, grossly, light-colored patches were found in the myocardium. Histologically they consisted of fibroblastic and myxomatous tissue with lymphocytes and large endothelial cells. Obliterating endarteritis was not uncommon. There were foci of small cell infiltration. The presence of the spirochetes was demonstrated by the Levaditi stain.

Billings in 1911 stated that in syphilis an obliterating endarteritis might especially involve the coronary arteries. Such a condition of the coronary arteries frequently results in fibrous myocarditis and the development of cardiac inadequacy and anginal attacks in the young. Sudden death may supervene.

Warthin in 1912 stated that in syphilitic hearts focal or diffuse areas of fatty degeneration of the myocardium may be associated with the presence of numerous spirochetes, without reaction on the part of the interstitial tissue. Such changes probably represent "a very acute or mild (latent) infection." They occur frequently in congenital syphilis. Calcification or fibroid changes may follow the parenchymatous changes, without a definite interstitial inflammatory reaction. In

such cases the spirochetes are few or have entirely disappeared, according to the stage of the secondary process. Warthin mentioned that focal areas of fatty degeneration of the myocardium also occur in other infectious diseases.

Benda in 1913 remarked that the seat of syphilitic changes in the heart is the myocardium. The changes may appear in the form of diffuse syphilitic inflammation, of fibrous inflammation or of gummatous inflammation and granulation tissue. In the discussion of Benda's paper, it was brought out that every author had confessed that he was unable to find specific signs of syphilitic myocarditis. Other diseases might cause similar changes in the myocardium. Fibrosis following gumma has nothing to do with syphilitic myocarditis.

Heller in 1913 could not decide whether the changes found in the hearts of 35 syphilitic patients were syphilitic. He was inclined to believe, however, that they were syphilitic in origin.

Brooks in 1913 reported that in 44 of 50 cases of syphilis there was a diseased myocardium. True gummas were found in 5 instances. The inflammatory process in the myocardium consisted of round cell infiltrations around the arterioles or of foci of fibrosis. The probable sequelae of such changes are cardiac aneurysms, which were present in 3 cases. A disease of the coronary arteries was found in 35 cases; the changes were of a relatively greater degree than the general arterial changes. In 5 cases in which the changes were pronounced, the age of the patients was below 30 and in 4 others below 40. Brooks maintained that cardiac involvement might develop early in syphilis.

Braun in 1913 mentioned that involvement of the myocardium might occur early in syphilis. The diagnosis, however, is based almost entirely on the anamnesis. In the late stages the heart may show syphilitic myocarditis leading to chronic myocardial insufficiency clinically. The author did not mention any cases of his own, but reviewed some reports in the literature on this subject. He pointed to the fact that in the textbooks of Bamberger, Zetelmayer, Friedreich and von Duck no mention is made of syphilitic myocarditis.

Sachs in 1913, reviewing the literature on this subject, remarked that some of the investigators were of the opinion that syphilis is the most important factor in the production of heart disease.

Rosenfeld in 1914 described a case in which scars were found in the papillary muscles of the heart. There was, besides, syphilitic aortitis with encroachment into the mouth of the left coronary artery and with almost complete obliteration of the mouth of the right coronary artery. The author concluded that pathologically syphilitic fibrous myocarditis cannot be distinguished from the usual fibrous (nonsyphilitic) myocarditis.

In 1914, Warthin, in one paper, reported 41 cases of active syphilis in 36 of which active lesions in the heart were found. He stated that syphilis was determined either by the presence of spirochetes or by the characteristic lesions of the tissues. He stated that in the latter the spirochetes were being demonstrated as fast as the work could be carried out. Warthin's other paper of 1914 will be referred to later.

Saltykow in 1914 reported a case of specific productive myocarditis. He described pale yellow areas in the myocardium. Histologically, granulation tissue was found, with many giant cells. There were necrotic portions, a few round cells and many endothelial cells. No tubercle bacilli and no spirochetes were found. The author was not certain that this was a case of syphilis.

Cabot in 1914 classified 600 recent cases in a hospital. Thirty-two of the patients showed failing hearts. These hearts showed partial valvular lesions. There was no evidence of nephritis or of arteriosclerosis, nor had any history of rheumatism been obtainable. But all these patients had given a positive Wassermann reaction. The author stated that the permeation of the myocardium of the congenitally syphilitic person with spirochetes makes it probable that in adults many cases of myocarditis are due to syphilis.

Brooks and Carroll in 1914 maintained that the heart may be affected in early syphilis. These authors, however, did not base their evidence on postmortem studies, but on clinical evidence.

Cesa Bianchi in 1914 described the case of a man, aged 39, who gave a history of syphilis of fifteen years' duration and a history of a rheumatic infection of two years' duration. The myocardium in the upper portion of the left ventricle showed a gelatin-like, grayish-white region, which on section showed new formation of connective tissue and of blood vessels, edema and perivascular infiltration by small cells. The periphery of this area showed giant cells and many plasma cells. There was no caseation or necrosis. The author spoke of granulomas. There was endarteritis of the small arteries. The finding of spirochetes was reported. The original article, however, shows no picture of the spirochetes. It seems likely that this case should be classified as one of gumma, rather than as one of syphilitic myocarditis.

Anders in 1915 remarked that involvement of the myocardium is not infrequent in the course of syphilis. While endarteritis is a frequent important lesion, actual gummas of the myocardium are distinctly infrequent. The author stated that, while he would not go so far as to say, along with some recent investigators, that syphilis is the principal factor in the production of heart disease, it can at least be safely assumed that rheumatism and syphilis head the list as causes of organic injury to this organ. He further stated that it is definitely known that the spirochete of syphilis has a selective action on the heart. But

neither the cause of the selective action nor the source of the information is revealed in this article.

Allbutt in 1915 remarked that the combination of syphilis of the heart and syphilis of the aorta is somewhat infrequent.

Thorel in 1915 maintained that, contrary to clinical observations, pathologico-anatomic experience teaches that cases of myocardial syphilis are extremely rare.

Citron in 1916 stressed the point that spirochetes are found in the heart in cases of congenital syphilis, but that disease of the heart is rare. Whether fibrous myocarditis results from localization of spirochetes in the interstitial tissue is not certain. This surely is, at least, not an everyday finding.

Symmers in 1916 reported anatomic lesions of syphilis in 314 of 4,880 cases in which autopsies were performed. Syphilitic aortitis was reported found in 175 cases, but changes in the myocardium were not mentioned.

Warthin in 1916 found active syphilitic lesions and spirochetes in the heart in 36 of 41 cases of syphilis. He did not mention the criteria of syphilis of the myocardium.

Hirschfelder in 1918 mentioned syphilitic fibrous myocarditis, without giving details.

Moore in 1918, in a clinical paper, ventured the opinion that cardiac lesions occur earlier in syphilis than has been thought. The cardiac lesions are most frequently present in the form of myocarditis. He did not say how he reached this conclusion.

Warthin's article of 1918 will be referred to later.

Aschoff in 1919 spoke of diffuse interstitial myocarditis with the formation of giant cells. He did not discuss any other form of syphilitic myocarditis.

Pilz in 1920 reported a case of constriction of the mouths of the coronary arteries in syphilis, resulting in necrosis, infarction and final rupture of the heart. He did not mention syphilitic lesions of the myocardium.

Sternberg in 1920 said that syphilitic arteritis of the coronary arteries may lead to circumscribed myomalacia, with the formation of scar tissue and possible rupture of the heart. He did not mention syphilis of the myocardium.

Takata in 1920 described 4 cases of gummas in the myocardium and 1 case of possibly syphilitic myocarditis. This case showed small, circumscribed cellular infiltrations close to the smallest blood vessels, mainly of round cells and plasma cells. There was new formation of connective tissue. Pigment was present in polymorphonuclear leukocytes. Some of the smaller arterioles and venules showed thickened

intima, while the larger arteries appeared normal. The veins showed circumscribed infiltrations by plasma cells; none were found in the arterioles.

Cowan and Rennie in 1921 discussed syphilitic aortitis and syphilitic aortic incompetence. In 3 of their cases the lumen of the left coronary artery was thrombosed. These cases showed myocardial infarctions. The authors presented 4 cases in more detail. None of them gave evidence of syphilitic myocarditis.

Chapman in 1920 maintained that in any case of organic heart failure in which the history of a more usual infection is not obtainable syphilis should be considered the causative agent. This is entirely a clinical paper, not confirmed by observations at autopsies.

Lemann and Mattes in 1920 reported an investigation carried out on 55 bodies showing syphilitic aortitis. Forty-one hearts were normal, on gross inspection, and 16 of these proved, on histologic examination, to be free from changes. Seven hearts showed scars in the myocardium, 5 offered areas of softening, 4 were the seat of fresh pericarditis (2 of these were from bodies that showed pneumonia), and in 1 heart an old pericarditis was demonstrable. Localized and diffuse areas of fibrosis were found in 14 hearts, whereas lymphocytic infiltration was present in 24. In 2 hearts spirochetes were found. One of these hearts showed no other abnormalities. Fatty degeneration of the cardiac muscle was found in 11 hearts; necrosis of the muscles, in 2. Sub-epicardial collections of lymphoid cells were demonstrable in 2, brown atrophy in 5 and simple atrophy in 3 hearts. Atrophy, necrosis and hemorrhage were found in 1. One heart was the seat of a microscopic abscess (the case showed other signs of clinical sepsis, but no other observations at autopsy were revealed). Two hearts showed gummas microscopically. The authors did not intend to suggest that all of these various changes in the cardiac muscle were due to syphilis. Certain changes, however, were suggestive. Scars and areas of softening, central necrosis of muscle tissue with connective tissue infiltration and beginning vascularization at the periphery of the lesion and lymphoid infiltration in the areas of vascularization were often observed. The end-picture of some of the lesions, namely, scarring with connective tissue replacement and diffuse infiltration by lymphoid cells, would make one suspicious of a healed gumma.

Macfie and Ingram in 1921 reported 3 cases occurring in boys from 6 to 12 years old. There was a rupture of the heart in 2 cases, and an old aneurysm in 1 case. The heart in 1 case showed a gumma-like structure; the other 2 cases offered a slight endarteritis of the coronary arteries, which possibly might be of syphilitic origin.

Spalding and von Glahn in 1921 reported a case of syphilitic rupture of the papillary muscle of the heart. Microscopically there were areas of necrosis surrounded by leukocytes. Some of the muscle fibers contained fat droplets. The Levaditi stain showed a moderate number of spirochetes. The spirochetes reproduced in the illustration of this article are not typical spirochetes. The histologic changes differed from those in a gumma, no plasma cells being present. The outlines of the preexisting tissue in the areas of coagulative necrosis were completely obliterated.

Brooks in 1921 stated that syphilis in the early and later stages involves the heart with great frequency. Syphilis may involve the pericardium, myocardium and endocardium and the conus arteriosus. Any form of syphilitic lesion, with the exception of the chancre, may be found in the heart. He believed that there are many cases of syphilis that show no anatomic evidence of cardiac involvement, notwithstanding that the heart is probably one of the most frequently involved viscera. The author gave the following data, without going into the microscopic details or even discussing differential diagnostic features: Among 50 cases, 35 showed coronary arteritis of a relatively greater degree than that found throughout other blood vessels. Because only one circumscribed area of the coronary artery might be involved, such an area might easily be overlooked by investigators. In 5 hearts gummas were found, usually of relatively small size. Syphilitic cardiac fibrosis originating from gummas was present in 4 cases. In 5 cases fibrosis and fatty degeneration and infiltration could be demonstrated. Syphilitic myocarditis was found in 6 cases (but no criteria for this diagnosis were given). In 3 of these cases there were cardiac aneurysms. The essential lesion leading to the aneurysmal formations was thought to be a coronary endarteritis.

Reid in 1922 stated that involvement of the heart may begin before or during the so-called secondary stage of syphilis. The infection of the heart and aorta by the spirochetes of syphilis produces one of the most important types of heart disease. In recent years there has been an appreciation of the fact that coincident with the syphilitic lesion of the aorta, the heart proper is usually involved. He further mentioned that sclerosis of the larger branches of the coronary arteries is rare in syphilis.

Borst in 1922 mentioned the occurrence of syphilitic scars in the myocardium. He stated that an aneurysm of the heart brought about by coronary sclerosis or thrombosis rarely leads to rupture of the heart, contrary to the aneurysm that is found as a result of syphilitic scars of the myocardium.

Wiltshire in 1922 made a statement in regard to diffuse interstitial syphilitic myocarditis as follows: "This is seen when the process has



been generally distributed about many of the small branches of the coronary artery. A diffuse fibrosis also is produced when the whole heart wall has been relatively starved over a considerable period by lesions causing narrowing of the openings or main trunks of the coronary arteries." The author did not cite any case of his own in which an autopsy had been performed, nor did he give a detailed histologic discussion.

Kaufmann in 1922 stated that not all cases of fibrous myocarditis in syphilis can be explained on the basis of vascular changes, but that cases are found in which toxic degeneration of the muscle fibers has occurred primarily and the proliferation of connective tissue secondarily. On the other hand, primary interstitial fibrous myocarditis has occurred in syphilis, although it is not characteristic.

Fraenkel in 1923 stated that there is no anatomic material proving the occurrence of syphilitic heart disease in the secondary stage of syphilis. As concerns the last stage of syphilis, the author differentiated between gummas of the heart and interstitial myocarditis. He stressed the point that the latter has no characteristic features.

Reid in 1923 stated that syphilis of the heart and aorta maims and kills those in the prime of life. Of 100 patients of the Boston City Hospital who successively came to autopsy, 7 showed syphilis to be the apparent cause of the changes in the heart and aorta. The author did not give the histologic details. He stated that every case of syphilis is a case of potential heart disease.

Levine, in discussing Reid's paper, asked those present at the meeting the following question: "What is the consensus of opinion as to the occurrence of syphilitic myocarditis without other vascular changes due to syphilis?" No one had any definite opinion as to the frequency of such occurrence, but the general feeling was that uncomplicated syphilitic myocarditis is rare.

Bloch in 1923 differentiated between acute syphilitic myocarditis, which is a true inflammation of the myocardium, and subacute myocarditis, which consists of a perivascular infiltration of embryonal cells, and which reveals an abundance of connective tissue and sometimes gummas. He stated that there is no pathologist who is able to make the diagnosis of syphilis with certainty ("d'une coupe"). The histologic examination cannot confirm the syphilitic nature of the lesion (quoting Nicolas and Moutot). Only the finding of spirochetes assures one that the lesion in question is syphilitic.

Wittgenstein and Brodnitz in 1924 stated that of 1,686 patients with cardiac and circulatory diseases, 542 were syphilitic. In 29 cases the diagnosis was inadequacy of the cardiac muscle, myodegeneration cordis, myocarditis or gummas of the myocardium. The diagnoses were based entirely on clinical findings. No case in which autopsy was performed was included in this report.

Von Glahn and Wilshusen in 1924 reported 2 cases of syphilitic aortitis combined with rheumatic myocarditis.

Scott in 1924 mentioned that among 25 patients showing syphilitic aortitis with involvement of the aortic valve post mortem, 8 showed the combination of fibrosis, cellular infiltration and mucoid degeneration, comparable to the histologic picture of syphilitic myocarditis described by Warthin. Cicatricial types of fibrosis were found in 4 of these cases. This was associated with demonstrable intimal sclerosis of the smaller arteries in 1 case only, in which infarcts were also present. Scott concluded that it seems unwarranted to attribute these changes to latent myocardial syphilis.

Hines in 1924 reported a case of syphilitic mesaortitis and myocarditis in a patient 32 years old. Grossly the myocardium showed recent and old infarcts and multiple thromboses of the small branches of the coronary arteries. The heart was hypertrophic. Histologically there were necrosis of the cardiac muscle fibers, granulation tissue with newly formed capillaries and many polymorphonuclear leukocytes. Perivascular infiltrations of lymphocytes predominated. Occasionally, fibrous scarring was noted. By the use of the Levaditi method spirochetes were found in the inflammatory areas and around blood vessels. He stated that although these striking myocardial changes are not rare, it is not common for syphilis to produce coronary thrombosis and infarction.

Howard in 1924 stated that in the late stage of syphilis a purely syphilitic myocarditis is not uncommon. In the earlier stages, however, parenchymatous degeneration and proliferation of lymphoid and endothelial cells about branches of the coronary artery and about the vasa vasorum prevail. This paper is based on clinical findings, without postmortem or microscopic observations.

Clawson in 1924 stated that syphilis of the myocardium appears to be rare. Of 9 cases of syphilitic aortitis, 3 showed myocardial fibrosis, and in 2 of these the condition seemed to be the result of coronary sclerosis.

Warthin, in discussing Clawson's paper, said that in his opinion Clawson's failure to find evidence of syphilitic myocarditis lay in the insufficient number of sections cut from the myocardium.

Moenckeberg in 1924 stated that Virchow's simple fibrous form of cardiac syphilis has no characteristic features. Such myocarditis is similar to scars formed as sequels to vascular disturbances. Moenckeberg spoke of gummas and of gummatous myocarditis with giant cells.

Palaase and Despeignes in 1924 reported a case of syphilitic aneurysm of the aorta. The myocardium showed no gross lesions. But the authors believed that they were dealing with syphilitic myocarditis and stated that they would refer to it subsequently.

Vaquez in 1924 classified syphilitic myocarditis as acute and sub-acute. Acute syphilitic myocarditis is evidenced through an extensive parenchymatous degeneration rather than a true inflammation. Sub-acute syphilitic myocarditis shows a diffuse, but slight, sclerosis and an interstitial infiltration by round cells which sometimes form miliary gummas around the blood vessels. The fibers of the cardiac muscle may show fatty degeneration or atrophy, or may be fragmented. The interstitial lesion is always associated with endoperiarteritis.

Stadler in 1925 said that interstitial syphilitic myocarditis leads to a slowly progressing, noncharacteristic inadequacy of the cardiac muscle.

Romberg in 1925 gave it as his opinion that damage of the heart in syphilis is not rare, but is rarer than damage of the aorta and coronary arteries.

Dietrich in 1925 stated that anatomic changes in the heart in syphilis are rare. Gummas may be found in the heart in syphilis, or scars as the result of gummas. Sometimes vascular changes are present. Whether the scars are really caused by the syphilitic agent or by something else cannot be decided. Ischemic conditions in other diseases may produce similar scars. The author did not mention the occurrence of a true syphilitic myocarditis.

Lenoble in 1925 stated that there is an interstitial form of syphilitic myocarditis which is characterized by the absence of calcareous deposits. A parenchymatous syphilitic myocarditis also exists, which occurs independent of vascular changes. It is possible that spirochetes either disappear quickly from such areas or do not exist there (*myocardites déshabitées*). In another paper he mentioned that one of the characteristics of syphilitic myocarditis is its rarity. This is in marked contrast to the frequency of syphilitic aortic lesions.

MacLachlan in 1925 pointed out in regard to a case of syphilitic aortitis which terminated in sudden death, and which was not given postmortem examination, that the sudden death could possibly be explained by the involvement of the coronaries, although the sudden death due to myocardial changes described by Warthin must be remembered.

Young in 1925 described an aneurysm and a gumma in the same heart. No atheroma was found in the coronary artery—a fact which induced the author to conclude that the aneurysm was of syphilitic origin. The left coronary was patent, but at its commencement the opening was flattened. He gave no histologic details.

Leschke in 1925 stated that diffuse interstitial syphilitic myocarditis is not rare in congenital syphilis. Later, however, it becomes rare. Sudden death is not uncommon in syphilitic myocarditis.

Warthin's paper in 1925 will be referred to later.

Guerich in 1925 wrote that of 23,179 autopsies, 806 showed syphilitic changes. Myocardial changes, however, were not mentioned.

Arnett in 1926 in a clinical paper mentioned that there is good reason to believe that during, if not before, the appearance of the secondary rash in syphilis, the spirochetes invade the cardiovascular system. But serious cardiovascular complications during the early stages of syphilis are extremely rare.

Karsner in 1926 said that syphilitic lesions of the heart may be represented either by gumma or by a diffuse chronic interstitial myocarditis. According to Warthin, the latter is extremely common in late syphilis.

Strauss in 1926 observed that syphilis of the myocardium is seen in the form of scattered patches throughout the muscle. The author did not go into histologic details.

Boyd in 1926 reported a case of acute myocardial syphilis. Histologically, lymphocytes and plasma cells were found here and there in relation to blood vessels. Similar collections of cells were present in the adventitia of the left coronary artery. In addition, polymorphonuclear leukocytes were found. The author claimed to have found spirochetes (the pictures of the spirochetes, however, are not typical of *Spirochæta pallida*).

Warthin in 1926 reported cases, and reached conclusions, similar to those in the paper of 1925.

Price in 1926 stated that fibrosis of the myocardium in syphilis may be the result of coronary disease or of gummas. The author continued:

Apart from inducing secondary fibrosis in the manner described, may syphilis give rise to fibrosis of the myocardium independently? In other words, is it among the causes of primary chronic myocarditis, i. e., a true inflammation? There is no doubt that syphilis may give rise to a primary chronic inflammation elsewhere; and it would appear that congenital syphilis does so in the case of the myocardium. Some authorities are of the opinion that acquired syphilis may cause a patchy or a diffuse fine fibrosis independently of the coronary arteries, while others are not. In any case, the histodiagnosis is very difficult even in the early stage so that it is probable that many of the cases of fibrosis of the myocardium in elderly people which are thought to be the result of syphilis are not so.

Delafield and Prudden in 1927 found that syphilitic myocarditis is accompanied by growth of connective tissue or by granulation tissue in the wall of the heart between the muscle fibers or in the walls of the blood vessels.

Kirch, in 1927, stated that the possibility of a primary fibrous myocarditis in syphilis is not proved. The syphilitic origin of the changes found in fibrous myocarditis is only probable. Spirochetes have not been demonstrated in such lesions.

Clawson and Bell in 1927 studied the hearts of 28 persons with syphilitic aortitis showing syphilitic involvement of the aortic valve. The hearts were studied carefully. Fibrosis was noted grossly in 1 heart. This heart also showed slight thickening of the coronary arteries, of the senile type. Microscopic areas of fibrosis were seen in 11 of the 28 hearts. The fibrosis was present in slight degree in all but 1, in which it was of moderate degree. The left coronary artery in this case showed a severe amount of senile sclerosis. The type of fibrosis was purely atrophic in 8 of the 11 positive cases. It consisted of atrophy of muscle fibers with replacement by scar tissue, without the presence of leukocytes. This atrophic type of fibrosis is the kind commonly found in old people with coronary injury. In 3 of these 8, slight gross injury was noted in the coronaries. In 3 the fibrosis was proliferative and of slight degree and might easily have been syphilitic in origin. Ten blocks from various parts of the heart of each of the 28 patients were stained for spirochetes by the Levaditi method. With each group of material, a tissue known to be syphilitic was stained as a control. This control material in every case showed many spirochetes, but spirochetes could not be found in any of the 28 hearts having syphilitic aortic insufficiency. The hearts of 15 patients who died rather suddenly were also studied. These hearts showed in addition to syphilitic aortitis a narrowing or a closure of the mouths of the coronary arteries. Gross myocardial fibrosis was not observed in any of these 15 hearts. Infarcts commonly found in cases of senile coronary disease were not present in any of the hearts which had syphilitic narrowing of the coronary orifices. Microscopic myocardial fibrosis was seen in 4 of the 15 hearts, three times in a slight degree and once in a moderate degree. The scars in all 4 were proliferative with lymphocytic infiltration. Ten blocks from each of 13 of these 15 hearts were stained for spirochetes and compared with a known control. Spirochetes were not found in any of the 13. The hearts of 23 patients who died from ruptured aneurysm showed no gross fibrosis. In 4 hearts a slight degree of proliferative fibrosis with lymphocytic infiltration was detected microscopically at the base of the heart. Spirochetes were sought in 22 of these hearts, as in the preceding hearts, but none was found. In 3 instances multiple gummas were found. Of 9 hearts from persons in whom syphilitic aortitis was found accidentally at the autopsy table, none showed gross myocardial fibrosis. Microscopic myocardial fibrosis was present in 3 of the 9 hearts, once in a moderate degree and twice in a slight degree. The fibrosis was atrophic in all.

Morris in 1927 mentioned gummas as sources of cardiac aneurysms in addition to syphilitic changes of the coronary arteries, but he did not give details of the latter changes. Syphilis of the heart assumes

in no way the paramount rôle that the spirochetal infection plays in the production of aneurysms of the aorta.

Henry in 1927, in a clinical note, stated that patients whose chief complaints are attacks of pericardial pains and arrhythmia, and who have low blood pressure, low hemoglobin and a one plus or negative Wassermann reaction, are not by any means all syphilitic, but that if no definite pathologic condition is found, it is well to make a therapeutic test.

Heimann in 1927 stated in a clinical analysis of 105 patients that syphilitic myocardial degeneration may occur without valvular mischief and sometimes without thickening of superficial arteries.

Groedel in 1927, in a roentgenologic study, maintained that in syphilitic disease of the cardiac muscle the relatively slight enlargement of the heart is often remarkable. But no proof is brought forward that the cases the author was dealing with showed syphilitic changes in the cardiac muscle.

Scott in 1927, basing his opinion on 75 cases that at autopsy showed syphilitic involvement of the aortic valves, stated that the histologic changes in the myocardium are not characteristic and to regard them as evidence of latent myocardial syphilis is unwarranted.

Schlesinger in 1928 ventured the opinion that in acquired syphilis the myocardium does not show the presence of spirochetes, contrary to the findings in congenital syphilis. He mentioned that disease of the myocardium in syphilis is common.

Lamb in 1928 was convinced that the myocardial changes in syphilis are really changes of great importance. To explain why these changes are not found more frequently, he mentioned that at autopsy it is unusual to take more than one or two pieces of cardiac muscle for study. Under these circumstances only the grossest lesions are seen, and the slighter changes are encountered by chance only.

Harris in 1928, in a paper based on observations of 100 patients (postmortem observations are not included), stated that as a matter of fact syphilis is a common cardiac disease, but unfortunately in the majority of cases not recognized.

To Clawson in 1928 it seemed evident that, even in a slight degree, myocarditis with syphilitic aortitis is rare, and that death, except in a few cases with myocardial gummas, is seldom if ever due to a myocardial inflammatory condition or to myocardial scars resulting from inflammation. He added that proliferative or exudative inflammation in the myocardium is rare in cases of syphilitic aortitis.

Kuelbs in 1928 stated that syphilis of the heart occurs either in the form of a diffuse gummatous interstitial inflammation or in the form of circumscribed gummas. Sclerotic foci with round cell infiltra-

tions, giant cells, gummas, periarteritis, endarteritis and endophlebitis are in the foreground.

Hazen in 1928 declared that it is becoming a matter not only of clinical, but also of autopsy record that the heart is frequently involved in syphilis. He often quoted Warthin, but did not offer cases of his own.

Reid in 1928 said that in recent years there has been an appreciation of the fact that coincident with the syphilitic lesion in the aorta, the heart proper is usually involved. Grossly, dilatation, hypertrophy, atrophy and patches of fibrosis in the wall of the left ventricle are often noted. He quoted Warthin frequently.

Arnett in 1928 described 2 cases of early syphilis in which death occurred from septicemia. He could not demonstrate spirochetes in the hearts. The sections also showed nothing characteristic of syphilis. He stated, however, that the changes in the myocardium due to the septicemia might have interfered with fine pathologic changes that could have been attributable to syphilis. He was unable to demonstrate organic cardiovascular disease in 25 patients afflicted with syphilis in the secondary stage.

Krumbhaar in 1928 remarked that syphilitic interstitial myocarditis probably occurs much more frequently than is generally supposed, but that unfortunately it is often difficult or impossible to differentiate syphilitic fibrosis from other forms of fibrosis.

Cookson in 1929 reported a case of cardiac syphilis with ventricular aneurysms occurring in a woman 43 years old. The heart showed a pale, slightly softened myocardium. Three aneurysms occupied the posterior part of the base of the left ventricle. On section it was noted that the walls of the aneurysms consisted of fibrous tissue containing considerable deposits of calcium. The valves were normal. There were slight, atheromatous changes in the aorta. A branch of the circumflex division of the left coronary artery was obliterated. Histologically, the aneurysmal wall showed necrotic tissue, bounded by dense fibrous tissue, which was infiltrated with plasma cells and lymphocytes. The plasma cells predominated. The blood vessels in the degenerated area showed marked obliterative changes, and, in some instances, the lumina were occluded. In spite of the finding of coronary sclerosis with obliteration of one of the coronary branches, the case is reported as one of cardiac syphilis.

Benson in 1929 described the case of a man, 66 years old. There were mild syphilitic changes in the aorta. The coronary ostia were patent, but the coronary arteries were sclerotic. The myocardium showed areas of necrosis and semitranslucent strands of fibrosis. The heart was the seat of a ruptured aneurysm. Histologically, lymphocytes were found in the adventitia of the coronary arteries. Their

lumina were almost completely occluded by a fibrous thickening of the intima. The media was infiltrated by lymphocytes. Atheromas and calcification were almost lacking. The myocardium showed necrotic portions. A perivascular infiltration of the interstitial tissue was not prominent. The necrotic portions in the myocardium were called gummas. The author believed that his case was similar to the cases of syphilitic myocarditis described by Warthin.

Cowan and Faulds in 1929, in a paper on syphilis of the heart and aorta, held that syphilitic endarteritis of the adventitial vessels of the aorta and of the arteries of the heart plays an important part in the causation of aneurysms and of ischemic fibrosis of the myocardium. There are several specific myocardial lesions, but in the majority of cases the myocardial lesions are only side issues, the result of coronary disease and so in no way specific. Diffuse fibrosis (subacute interstitial fibrosis) has been found during the secondary stages in the adult, and more often in congenital syphilis in infants and young children. As a result of occlusion of the orifices of the coronary arteries or of narrowing in their course by specific endarteritis, an infarct may occur or a para-arterial fibrosis may result. The ultimate lesions in the muscle are not syphilitic, though they have a syphilitic cause.

Lukomski in 1929 stated that the damages of the aorta and heart in late syphilis are the results of vascular changes in early syphilis.

Levine in 1929 remarked that syphilis is not a common cause of coronary thrombosis. Four and one-half per cent of the cases of coronary thrombosis were syphilitic. But it does not follow that even in these syphilis had a direct causative influence.

Simpson in 1929 pointed out that there is a lack of agreement as to what constitutes the histologic picture of cardiovascular syphilis. He was of the opinion that syphilitic myocarditis is a common disease. He stated that identification of spirochetes in the tissue furnishes conclusive evidence of the disease.

Templeton in 1929 remarked that myocardial changes are due to coronary blockings, and that the whole myocardium may be actually involved by the spirochetes.

Gravier in 1929 classified syphilitic myocarditis as mechanical lesions brought about by an obliteration of the coronary arteries and as inflammatory lesions (myocarditis, subacute and chronic). The author described the case of a woman 44 years old. She presented syphilitic aortitis and an enlarged heart with open mouths of the coronary arteries. The myocardium, grossly, revealed no changes. Histologically, the myocardium showed an interstitial connective tissue reaction, with marked inflammation and edema. Many plasmacytes were spread between the muscle fibers. The capillaries were apparently dilated. Other fields showed greater dissociation of the muscle fibers, and the



connective tissue was more marked. The cellular infiltration here was more or less pronounced. Other portions revealed that the connective tissue had not only spread apart the muscle fibers, but also had formed actual bands of fibers. There were many capillaries in these fields, but only a few of the arterioles were the seat of endarteritis. The author stated that subacute syphilitic myocarditis may show large fibrous lesions grossly, but that more often the lesions are detectable only by the microscope.

Brugsch in 1929 made the statement that the results of the studies of Grassmann, who found abnormal heart rhythms in 15 per cent of 288 patients with early syphilis, do not indicate a serious involvement of the heart. In the late stage of syphilis involvement of the heart is rare as compared with involvement of the aorta. Syphilis may present itself in the form of myocardial scars resulting from syphilitic arteritis of the coronary branches, or it may produce the myocardial lesions directly.

Wilson in 1929 described the case of a man 44 years old, with clinically a complete heart block. The heart with the aorta weighed 940 Gm. The papillary muscles were enlarged and sclerosed. The coronary vessels were enlarged, their walls thickened. The aorta showed syphilitic aortitis. The cardiac muscle, histologically, revealed patchy areas of sclerosis and an infiltration of round cells in the interstitial tissue. By the use of the Levaditi stain spirochetes were demonstrated in the interventricular septum. They were found just below the aortic valve. The author made the diagnosis of syphilitic myocarditis. Pictures of spirochetes are not shown.

Hajóshi in 1929 described the case of a patient whose heart, in the author's opinion, showed gummas. The case, however, showed, in addition, tuberculosis of the lungs and lymph nodes and a caseous tuberculous pericarditis.

Dennis in 1930 remarked that the myocardium in late syphilis shows rather constantly perivascular foci, identical with perivascular foci seen elsewhere in syphilis. A various degree of reparative fibrosis and its secondary effects can also be found. He believed that death results more particularly from syphilitic aortitis with its accompanying aortic insufficiency, from aneurysm or from coronary occlusion.

MacCallum in 1930 stated that there is a tendency to ascribe the final failure or decompensation of the heart in cases of syphilitic disease of the aorta and the aortic valves to a corresponding disease of the myocardium. He had seen few cases in which active syphilitic myocarditis was suggested, and he was unable to feel sure that scars found in the myocardium were syphilitic. Nevertheless, that syphilitic disease of the myocardium could occur in the form described by Warthin he thought was fairly self-evident, and that it would heal with the traces indicated by such scars was equally clear.

Warthin in 1930 described the case of a 24 year old man with extensive syphilitic myocarditis associated with malignant syphilis. The heart showed grossly a slight yellowish striping. The coronary arteries appeared normal. Histologically, there were fatty degeneration and infiltration of the myocardium, increase in interstitial nuclei, proliferation of fibroblasts, reticulo-endothelial cells and interstitial edema. There was infiltration by plasma cells and lymphocytes, showing a typical "single file" arrangement, characteristic of syphilitic myocarditis. Monocytes were also present and a few polymorphonuclear leukocytes. There was a perivascular aggregation of cells around smaller branches of the coronary vessels. The larger aggregations could be styled as miliary gummas, but in general the process was rather diffuse. The muscle fibers were as a rule atrophic. Fragmented spirochetes were seen, broken into portions with two or three spirals. A few perfect spirochetes were found, one in a giant cell.

Chanotis in 1930 reported the case of a man 47 years old. The diagnosis was syphilitic aortitis with diffuse sclerotic myocarditis. Microscopically, the heart showed diffuse connective tissue proliferation, with degenerative changes, and edema. The coronary arteries did not show changes that could explain the myocardial lesions. Chanotis therefore believed that his case was one of primary syphilitic myocarditis. It was called primary to differentiate this form of myocarditis from myocarditis secondary to vascular changes. He stated that if in such cases spirochetes cannot be found, the diagnosis of syphilis can be made by the existence of other clinico-anatomic factors.

Cowan in 1930 remarked that diffuse fibrosis, subacute interstitial fibrosis, has been found in the secondary stages of syphilis in the adult and more frequently in congenital syphilis in infants and young children. But the most important and the most frequent sequel of syphilis is due to syphilitic disease of the coronary arteries and ischemic fibrosis. The arteries may be narrowed or occluded at their orifices by a syphilitic plaque in the aorta, or in their course by a specific endarteritis obliterans. Infarct or para-arterial fibrosis results. The ultimate muscular lesions are not specific, though they have a specific cause.

Coombs in 1930, in an address on cardiovascular syphilis, dealt mainly with syphilitic aortitis and syphilitic aortic insufficiency. He mentioned that the coronary orifices are sometimes "nipped in a tightening grasp," the result being starvation of the muscles which they supply, with replacement fibrosis and degeneration of the myocardial fibers.

Coombs in 1930 also described hearts of persons showing syphilitic aortitis. Many hearts showed evidence of malnutrition. Often an increase in connective tissue was seen, with little or no inflammatory

reaction. In 1 heart, a granulomatous focus was observed. In some hearts, the author described a weak cellular response—a few plasma cells and lymphocytes—drowned in serum, which gave the tissue a dropsical appearance.

Clawson in 1930 mentioned that in rare instances syphilis causes gummas of the heart or diffuse exudative and proliferative inflammations within the myocardium.

Bell in 1930 stated that syphilis of the myocardium is rare. Patches of fibrous tissue in the myocardium are generally due to coronary disease; but syphilis is to be considered if there are large numbers of lymphocytes.

Martland in 1930 reported an investigation based on observations in the myocardium in 101 cases of syphilis of the aorta and heart, in which death had occurred suddenly, and in which autopsy was performed. The myocardium in these cases was usually normal or showed only slight or moderate hypertrophy. No evidence of specific myocardial lesions was noted. In a few cases, the heart was small, and the myocardium the seat of brown atrophy. In none of the cases was there any evidence of specific myocardial lesions sufficient to explain death. Any lesion in the myocardium, besides hypertrophy and occasional brown atrophy, is usually interpreted as due to superimposed arteriosclerosis of the coronaries. The author concluded that syphilis involving the heart in portions other than the region of the aortic valve is unusual and is not of great clinical or pathologic importance.

Carr in 1930 studied the gross changes in the heart in 119 cases of cardiovascular syphilis. He found that, except for a predominant left ventricular hypertrophy that resembles that of essential hypertension, the gross myocardial changes associated with syphilitic aortitis are not characteristic.

Maher in 1930 reported the microscopic lesions of cardiac syphilis in 5 cases of uncomplicated and probably untreated syphilitic aortic regurgitation. He stated that the inflammatory reaction, presumably due to syphilis by exclusion of other factors, was found to be a lymphocytic and plasma cell infiltration about the coronary arteries and their branches and beneath the visceral pericardium. In the muscular tissue, the invasion appeared between the fibers and could be demonstrated about the capillaries. In the discussion, the author stated that the coronary arterial picture is the same in syphilitic lesions as in lesions in other cases, namely, an infiltration by round cells about the vessels. He did not demonstrate spirochetes in the myocardium, although he thought that they were there.

Paullin in 1930 reported two cases of syphilitic myocarditis. The first case showed, microscopically, numerous areas of degeneration in which the cardiac muscle had been completely replaced by bands of

fibrous tissue, and there were numerous collections of lymphocytic infiltration scattered here and there throughout the muscle and about the arteries. Occasionally, fibroblasts and plasma cells were visible in these fields. The second case showed a marked infiltration by lymphocytes, plasma cells and polymorphonuclear leukocytes, with complete destruction of the cardiac muscle. There was also evidence of fibroblastic proliferation and edema. Other areas were noted in which there was an old fibrosis with replacement of the cardiac muscle by bands of connective tissue. Sections from this heart were examined by Dr. Warthin, who demonstrated spirochetes in the areas of syphilitic inflammation.

Moritz in 1931, in a paper on syphilitic coronary arteritis, did not mention an involvement of the myocardium.

*(To be concluded)*

# THE ETIOLOGY OF CANCER

## III. MISCELLANEOUS FEATURES \*

II. E. EGGERS, M.D.

OMAHA

### NUCLEAR ABERRATIONS

*Nuclear Fusion.*—With cellular reproduction forming such an outstanding part of the phenomena of cancer, and especially in view of the fact that nuclear abnormalities are such a conspicuous feature of its morphology, it was only to be expected that among the theories of the causation of cancer would be those explaining this by nuclear aberration. A number of such theories have been advanced, the principal ones of which would explain cancer either as the result of acquisition of abnormal properties by the cell as the result of nuclear fusions, or as being due to the loss by the nucleus of those factors determining regulation of growth. Of these two principal and divergent theories, that of nuclear fusion is the older. In 1887 Karg advanced the idea that the unlimited reproduction of cancer cells was to be explained by such a mechanism, and in explanation of their extreme capacity for migration with survival, he suggested that the other element entering into the fusion was the leukocyte, so that the offspring of this union showed not only features characteristic of the original cells of the cancerous tissue, but in addition to the newly achieved properties of excessive reproduction, those of ability to infiltrate and metastasize. Klebs shortly afterward promulgated quite independently the same theory, and Schleich almost simultaneously expressed the view that in nuclear fusion was to be found the explanation of malignant growth, but did not attempt to identify the cells contributing the added nuclear material, regarding these as being possibly at times those of another individual. Shattock and Ballance had already taken a somewhat similar view and had described in cancer cells karyorrhexis with extrusion of nuclear fragments, which they suggested might function in a manner similar to the fertilizing action of spermatozoa. Morpurgo in 1894 expressed the view that the intracellular inclusions, which about that time were exciting much interest because of their frequent identification as protozoan parasites, in reality represented chromatophil material which was attracted to dividing nuclei, a view essentially similar to that of Shattock and Ballance, although without the etiologic implications of the latter.

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In 1903 Farmer, Moore and Walker described heterotypic mitoses in cancer cells, with a reduction of chromosomes similar to that of sex cells, and Farmer observed what he regarded as evidences of fusion in such cells, while Bushnell suggested that their growth might rest on a parthenogenetic basis. The observations of Farmer and his colleagues received corroboration by Bushnell and Cavers, Bashford, and Bashford and Murray, and excited a considerable degree of interest, although their significance was variously interpreted. Campbell, for instance, regarded the change as one of reversion to a primitive type of cell characterized by having a reduced number of chromosomes—an idea of reversionary metamorphosis that was not particularly new as applied to cancer, as it had been advanced by Gresswell in 1887. Although Farmer, Moore and Walker described what they regarded as evidence of nuclear fusion in cancer cells, and particularly with nuclear material of leukocytic origin, they were careful to state that such evidence of fusion had not been found in connection with heterotypic cells, however attractive such evidence would have been in support of their theory. Bashford, basing his opinion on the fact that despite the recurrent presence of nuclei with reduced chromosomes, this reduction did not reach beyond one half of the normal somatic number, expressed the view that nuclear fusion would be necessary to keep reduction within these bounds. Other later advocates of the cellular fusion theory have been Hallion, in 1907, and Welsh, and Kotzenberg as lately as 1922. Practically all the writers cited previously took the view that the fusion was between cells of the same individual, but Kronthal, writing in 1906, considered that the fusing element was from another, and even more extreme speculations have been advanced by Evans, Shannon and Stefani, with the hypothesis that cells of parasitic protozoa were concerned. Aichel in 1911 advanced a fusion theory based on the relatively independent ground of the work done on sea-urchins' eggs by Boveri, in which multiple fertilization was found possible. An ingenious suggestion as to the cause of the varying properties of benign and malignant tumors was embraced in the theory as advanced by Aichel, to the effect that while the conspicuous properties of malignancy were the result of fusion of somatic cells with leukocytes, benign tumors were the product of fusion of somatic cells alone. Another rather ingenious variant of the cell fusion theory was that of Skerrett, who, not satisfied with leukocytic fusion as adequate ground for malignant behavior, would have these cells serving as carriers for germ cell idioplasm to the cells the fate of which was to become cancerous. It was not long after the earlier publications of Farmer, Moore and Walker that their results, or more particularly their observations, were questioned. Bashford and Murray in 1906 reversed their former corroboratory opinion; Dor expressed his doubt of the validity of their

observations, and in 1910 Howard and Schulz, and Deton, reported that they were unable to verify them.

*Nuclear Reduction.*—As opposed to the theory of nuclear excess, or of restoration to normal chromosome value by fusion, are the several theories that would explain cancer by loss of nuclear material, and so of factors determinant of normal cellular behavior. Strictly speaking, in large part these theories, and especially the anaplasia theory of von Hanseemann, are explanations of the mechanism of cancerous growth rather than of its origin. The first promulgation by von Hanseemann of his theory appeared in 1890; essentially it stated that in every asymmetric cell division there occurs an alteration in differentiation, with possible corresponding alterations of growth energy and growth direction. From asymmetric division two types of cell might result—those with fewer nuclear segments, capable only of limited further growth and reproduction, and others with unrestricted and actually unrestrained powers of further growth. As distinct from the theories that would regard the cancer cell as one showing embryonic reversion, von Hanseemann pointed out that cells showing this anaplastic change differ from embryonic cells, which are the possessors of undeveloped potentialities in the way of differentiation, as in the anaplastic cells differential potentialities are completely lost. The principal objections to von Hanseemann's theory have been on the grounds that asymmetric mitoses are not exclusively peculiar to cancerous tissues, Stroebe having reported their occurrence in normal regenerative processes and Krompecher in embryonic tissues, and Häcker having found that a number of agents might induce their appearance. But as pointed out by von Hanseemann, it would be only in the occasional case in which asymmetric division entailed the loss of the growth-regulatory mechanism without serious impairment of cellular viability, that malignancy would result. In many respects similar to the theory of von Hanseemann is that of Boveri, who likewise believed that the peculiarities of cancerous behavior could be explained by loss of nuclear material with corresponding loss of cell regulation. In experiments conducted on sea-urchins' eggs, Boveri found that such loss might be caused by irritants of the type known to cause cancer, thus adding an etiologic element to the theory which was absent in von Hanseemann's speculations. Rockey would explain irregular mitoses as the result of repeated interference with normal regeneration, along with cellular displacement, the two combined eventually leading to hyperkaryokinesis and distorted nuclear activity. An excellent summary and discussion of von Hanseemann's theory is that of Whitman, who in 1920 advanced the view that the cancer cell is a new variety of cell, which originates by somatic mutation through interference with mitosis—an idea essentially similar, though expressed in different terms, to that of Rockey—and views

similar to those of von Hanseemann have been published by Oertel, by Perthes and by Levy.

The view of cancer as the result of a reversionary metamorphosis, alluded to in connection with Campbell and Gresswell, was advanced by Gilchrist in 1909, and again by Campbell in 1920, with the added suggestion that it represents an abortive attempt at asexual reproduction similar to that of lower orders of animal life. The more usual view is to regard cancer as a reversion to a sexual type of cell; this was suggested in connection with the work of Farmer, Moore and Walker already cited, and Walker in 1905 called attention to the similarity of Plimmer's bodies to the archoplasmic vesicles seen in spermatogonia, again with the suggestion of phylogenetic reversion. And again in 1911 Walker and Whittingham called attention to the analogies between the nucleus of the cancer cell and that of the sex cell. A not dissimilar view, but with closer kinship to Cohnheim's hypothesis, is that of Beard, who, largely on the grounds of nuclear peculiarities in cancer, regarded neoplastic cells as resulting from the displacement of primary germ cells—a hypothesis that Grünbaum believed to be confirmed by the findings of Farmer and his co-workers.

Other attempts to explain the phenomena of cancer by various types of assumed nuclear aberration are: that of Fabre-Domergue, who would ascribe carcinoma to a lack of relationship of the planes of nuclear division to body surfaces, so that the epithelial cells no longer arrange themselves, nor regulate themselves, with relation to those surfaces; a somewhat similar theory of Forbes-Ross, who also regarded the fundamental change in the cancer cell as one of lost polarity; that of Jessup, who would regard cancer as due to external stimuli effecting a disturbance of intracellular electrical equilibrium, with resultant loss of nuclear symmetry. Ries believed that cancer resulted from nuclear injury; Ariens-Kappers suggested that malignant growth centered in centrosomic disturbance, since the centrosome is regularly to be found on the side most exposed to irritation; Lumiere would ascribe malignant growth to unduly prolonged exercise of the function of regenerative reproduction. Robertson and Heiberg believed that the excessive proliferative rate of malignant tissues is the result of a disturbance of the normal ratio between nucleus and cytoplasm, which Sokoloff found to be low in cancerous cells, the essential change, according to Robertson, being the production of cells capable of dividing at abnormally low relative amounts of nuclear material as compared with the amounts of cytoplasm. Heiberg, on the other hand, would regard the fundamental change as one of increase of nuclear volume, not necessarily associated with an altered nucleocytoplasmic ratio, since the volume of the cytoplasm might also be increased, but with an alteration of chromosome content by virtue of which the cells suffer a permanent



alteration of growth energy; in small cell tumors an actual increase in nucleocytoplasmic ratio, overloading the cells with nuclear material, would force them to rapid and sustained division.

To some extent, the relationships between nuclear irregularities and malignant growth have lent themselves to more or less experimental study, in connection with tissue cultures. In 1913 Lambert reported that atypical mitoses were to be seen in cultures of sarcomatous, but not in those of normal, tissues. Mottram, reporting on experiments with cultures of renal tissues of the normal rat, observed that with a normal carbon dioxide tension of 40 mm., nuclear division was frequent, but that with considerably higher tensions the mitotic figures became abnormal, with frequent irregular migration of the chromatin. Goldschmidt and Fischer in a study of nuclear division in cultured carcinomas from mice, published in 1929, found pronounced irregularities in the way of multipolar mitoses only very rarely, but the chromosome count in the great majority of mitoses was less than normal, ranging as a rule from 32 to 36, as compared with the normal somatic count for the mouse of 40.

As regards observations on excised tumor tissues, Evans reported that for any accuracy immediate fixation of the material is absolutely essential, and that if this is prompt, most nuclear figures will be found to be normal. In these circumstances he observed occasional tetrad chromosomes, but no reduction below the normal number. With the exception of this work, there is no evidence that can be regarded as contradictory to von Hansemann's theory of anaplasia; but this, as has been suggested, and as was acknowledged by von Hansemann himself, is not an explanation of the etiology of cancer, but rather of the mechanism through which malignant growth may be achieved.

#### NEUROTROPHISM AND CANCER

In connection with the subject of the tar cancers, reference has been made to possible influences of trophic nervous changes on cancerous development. As a matter of fact, such influences have been suggested as the actual determining factor in the causation of cancer. Lang in 1879 suggested that cancer might originate on such a basis—a suggestion that was renewed by Dieffenbach in 1906—while Hodgson in 1905 advanced the idea that persistent nerve fatigue was the cause, and Köhner in 1907 regarded cancer as resulting from the release of cells from central control, either nervous or humoral. Masani also, in 1911, ascribed the disordered growth of cancer to the lack of an organizing, regulating mechanism normally contributed through the nerves. Stajano in 1922 ascribed cancer to trophic causes, arguing from the relations between surface cancers and trauma, and the alleged

liability of cancer to develop in sites normally abundantly innervated. Engel would explain the action of cancerogenic chemical agencies by their action on the local nerves, and Molotkoff, on the basis of a very similar view, suggested neurotomy as a means of treatment, and believed that with this he was able to influence the course of cancer favorably. A less direct influence is assumed by Lorin-Epstein and Bondartschuk and by Willy Meyer. The former would interpret the influence of nerves in the development of cancer as in the nature of a general effect, acting in addition to definitely local cellular factors. The view of Meyer is not dissimilar, in that he regarded salt imbalance as one element in the causation of tumor, and believed that in part this may be achieved through sympathetic nervous disturbance. That there is a frequent correspondence between the growth area of superficial cancers and the distribution of sensory innervation has been particularly emphasized by Cheatle with reference to facial cancers.

As to the experimental study of cancer, this has contributed a certain amount of information, sometimes conflicting, as to the influence of innervation on the causation or progress of cancer. The possibility that nerve mechanism is fundamentally involved in the former can be considered as definitely excluded by the indirect evidence afforded by the experimental induction of malignant growth in conditions entirely free from nervous elements, as in the cultured tissues of Fischer and Laser. On the basis of observation of already initiated cancers, there is certainly no antagonism between innervation and cancerous growth. Begg found that not only may tar cancers invade nerve cords, but that these may actually afford ready paths for the farther growth of the neoplasm, and in a recent article Meissel and Larionow reported the effects of implantation of a transferable carcinoma of the mouse into a severed nerve cord. Not only was there an absence of antagonism between the two, but an actually redundant regeneration of nerve fibers occurred, with growth into the tumor to form plexuses about the tumor lobules, and with the penetration of single fibers even more intimately into the cancer. As to the natural relations of nerve fibers to malignant tumors, the testimony is not altogether concordant. Nakamoto and Tsunoda both reported an absence of nerves in tar cancers, and the former stated that in the course of development of these cancers there is a relatively early disintegration of the peripheral nerves. On the other hand, Itchikawa, Baum and Uwatoko, and Herzog, using principally silver impregnation methods, were able to demonstrate what appeared to be nerve filaments in the stroma of the tumors studied, and Oertel reported finding them within a number of carcinomas and sarcomas.

As concerns the effects of nerve lesions on the development of cancer, the work of Auler, Itchikawa, Kotzareff, Remond, Sendrail

and Bernardbeig, and Cramer, has been cited in connection with the tar cancers. Since their work, similar results have been obtained by Heim and Tinozzi, and Tsunoda, and recently by Eiger and Czarnecki, all of whom observed evidence that sympathetic innervation exerts a restraining influence on the growth or development of tumors. Hirsch-Hoffmann, too, has observed that the repeated injection of sympathetotropic ephedrine or adrenalin causes increased growth of tumors. That the effect, however, is principally achieved through altered nutrition and is less concerned with the induction of tumors, is suggested by the experiments of Aschner, who found the hyperemia that followed section of the nerve supply of the extremities accelerated the growth of tumors implanted there, and those of Pearce and Van Allen, who observed enhanced growth of transplanted neoplasms after cervical sympathectomy. As to the work of Molotkoff in which he reported favorable results after resection of nerves in human cancers, Ssokolow, with similar material, was quite unable to confirm it.

#### HEREDITY AND CANCER

Efforts to determine the effect of heredity on the incidence of human cancer have yielded results which at most must be regarded as indecisive. An analysis of parental histories made by Paget in 1866 showed among the parents of cancerous persons an incidence of that disease of 24.2 per cent, and in 1878 Cripps in a similar study found that practically identical relations obtained between the general incidence of the disease and that in which there was present the direct hereditary relationship—29 and 29.1 per cent, respectively. The incidence rates reported by Williams in 1884 were very similar, but they showed marked disparity in the hereditary element as concerned cancers of various organs, ranging from a frequency of cancerous parental history of 23.7 per cent in cases of uterine cancer to one of 3.3 per cent in cases of skin cancer. Hutchinson in 1886 reported the interesting observation, which has been supported by later observations, that in most cases of the occurrence of carcinoma at an early age, there is a definite history of ancestral cancer.

But while in general the analyses of the relations of cancer at least to immediate heredity failed to show any definite connection, there were collected a considerable number of instances of familial cancer so striking as scarcely to admit explanation on the grounds of coincidence. Among these is the famous example reported by Broca, of a cancerous mother all of whose daughters died of the disease, and with a total of 15 cases among 26 persons belonging to 3 generations. Other cases of generally similar character have been reported by Hardman, Bodilly, Fere, Rebulet, Power, Nason, Williams, Smith, Watkins and Jullien. In practically all of these the cancers were in part at least heterotopic.

although in the family described by Watkins there was a repeated occurrence of rectal cancer, and with this was associated a particular frequency of rectal disease in general. In the example reported by Fere, in which the cancers were practically entirely mammary in location, there was a definite association of agalactia. Although the majority of those making these early reports on cancerous families were firmly convinced of the hereditary character of familial incidence, this view was not generally accepted. Pearson in 1904 expressed the belief that the evidences of a hereditary relationship were so slight as to come within the bounds of random sampling; von Hanseemann took the view that at most there might be a barely possible element of hereditary predisposition, and Ledoux-Lebard regarded the instances of reported familial occurrence as purely fortuitous. In addition to data similar to the type cited, one or two studies tended to show what might be regarded as a racial distribution of cancer. Kruse in 1901 found a markedly greater rate of cancer mortality in northern than in southern Italy—a difference that he believed to be of racial origin—and Lyon in Buffalo made the interesting observation that cancer there was most frequent in families of Germanic stock. Madden reported that while cancer in Egypt is common among the Arabs and Copts, it is rare in Negroes. To what extent these racial differences may be due to differences in habit or in living conditions is not known.

In later years there have been reported a considerable number of instances of familial cancer. Warthin in 1913 reported a number of these, and commented on the fact that when in a single generation there occurred several cases of cancer, there was almost certain to be a history of ancestral cancer, and he confirmed Hutchinson's earlier observation that these cancers are likely to develop at an early age. Manson in the same year published an account of the associated occurrence of sarcoma in a mother and her 2 children, and Peiser in 1915 reported 2 instances of cancer families. More were reported by Peller in 1922, and Wassink in 1924 found that among 258 cases of mammary cancer, 76 were associated with similar cancers in other members of the same family. Kaiser in 1924 described a family with a marked predisposition particularly to gastric cancer; Letulle reported several cases of familial cancer, and Swoboda 1 instance. In respect to the number of generations traced, few of these accounts compare with that published by Warthin in 1925, in which, among the offspring of a cancerous great-grandfather, there was an incidence of malignant disease of 18 per cent of the offspring, or 31.8 per cent of those who had attained adult life. There was a marked tendency to gastro-intestinal cancer in the males and to cancer of the organs of generation in the females, with the strikingly early average age of incidence of 37.9 years. Ledo in 1927 described a family that showed an exceptional tendency

toward squamous cell cancers. On the other side of the picture are the families described by van Dam, in which in 12 instances traced for 4 or 5 generations there was no indication of cancerous inheritance, and the observation that in 15 families in each of which both parents had died of cancer, none had occurred among 45 children who had attained the usual age for the appearance of cancer.

While, in general, the data on human beings have left dark the matter of hereditary influences in the causation of cancer, there is a mass of accumulated data, obtained from observation of animals, that is of very definite character. Among the earlier instances of the occurrence of cancer in lower animals on an apparently hereditary basis was the observation by Eberth of spontaneous cancer in 3 offspring of a single pair of white mice, and that cited by Williams, of the occurrence of melanosarcoma in the descendants of a white stallion that had itself died of that disease; but this case might be explained by the peculiar relation that is known to exist between absence of pigmentation and the occurrence of these tumors in horses. In 1900 Loeb and Jobson reported the focal occurrence of bovine cancer on a Wyoming ranch, and in 1903 Voges described similar findings in the Argentine, with the added observation that these cancers occurred in pure white-headed Hereford cattle. Loeb took the view that such foci were to be explained on the basis of heredity, and suggested the same explanation for the so-called "cage epidemics" that were being reported from time to time among white mice.

The laboratory study of the relationship was first pursued from the point of view of susceptibility to implantation of tumor. In 1907 Tyzzer, in connection with an observed apparently hereditary tendency to the development of spontaneous tumors of mice under his observation, believed that this tendency was associated with an immunity to the transplantation of tumor, and Haaland observed that different strains of mice varied greatly in their susceptibility to the implantation of specific tumors; but he made the additional observation that in conditions of altered environment this variation tended to disappear, and eventually his imported mice behaved exactly like those of his original stock. Although, according to Gierke, the influence of heredity in this relationship was minor in effect compared with that of environment, Tyzzer was able, in a later paper, to show that at times liability to successful implantation of tumor was almost entirely a matter of inheritance. An investigation of this subject was made in some detail by Levin in 1911 and again by Loeb and Fleisher in 1912, and these observers agreed with Tyzzer that heredity exerted a marked effect—according to Levin, in accordance with mendelian laws; according to Loeb and Fleisher, if in accordance with mendelian principles, there is necessary an assumption of the existence of multiple factors. McFarland and

McConnell in 1913 likewise found that in different strains of mice there was varying susceptibility to the transplantation of different tumors, and in 1915 Tyzzer reported on a strain of Japanese waltzing mice in which only 0.39 per cent escaped in the course of 8 years of successive transfers, while common mice were completely insusceptible to the particular tumor used. In the first generation of hybrids only 5 per cent succumbed to the transplanted tumor, and later hybrid generations were almost completely immune. As to the relations of this hereditary insusceptibility to mendelian principles, Tyzzer, like Loeb and Fleisher, believed that this was a matter of factor-complexes. The relationship is certainly not a simple one, as Lathrop and Loeb found that at times hybrid animals may show a high, at times an intermediate, and occasionally a low, rate of susceptibility; Morpurgo and Donati observed that with a resistant stock of animals, the first generation of offspring of susceptible exceptions showed no higher incidence of "takes" than did those of the nonsusceptible animals—indicating for susceptibility the cooperation of other factors than those of hereditary character alone. In contrast with the findings of Lathrop and Loeb, and with those of Tyzzer, Lynch reported an instance of susceptibility to transfer that behaved like a mendelian dominant. Roffo reported an observation of rats in which susceptibility to implantation of tumor was associated with inherited color characteristics.

There is of course little evident relationship between the problem of the hereditary character of susceptibility to transfer and that of the causation of cancer, except to the extent that the inception of cancer would in part be dependent on factors that might be necessary to permit the survival of cells already become malignant. Even in this connection, the results obtained with introduced tumors of foreign origin would have to be interpreted with the reservation that as they do not represent the cells of the individual concerned, their relations may well be quite different from those of the host's own cells after these had become neoplastic.

Of much more direct significance are the experiments that bear directly on the hereditary transmissibility of a predisposition to cancer. Tyzzer, as has been mentioned, believed as early as 1907 that he had observed a distinctly hereditary tendency toward spontaneous tumors in mice, and although Bashford a year later believed that there was no definite evidence of such tendency shown either in the tumors of man or those of lower animals, Thorel in that year reported that he had seen the occurrence of 14 spontaneous tumors within 1 year among 60 mice of the same descent. More instances of "cancer families" in mice were reported by Tyzzer in 1909, and Cuenot and Mercier, dealing with this phase of the etiology of cancer, expressed the conclusion that while cancer in mice might be in part of hereditary origin, it was not

controlled by mendelian characteristics. In 1911 Bashford reported on experiments that reversed his former view, and Murray also described what was evidence of hereditary predisposition to cancer in mice. Henke in 1913 reported on an epidemic of cancer in mice, which appeared to rest definitely on a hereditary basis, and in that year Slye began her publications of the pedigrees of mice, which clearly showed the now undoubted relation between spontaneous tumors of mice and heredity. In 1914 she announced that of the 155 spontaneous tumors that she had observed in mice of which the ancestry could be traced, 146 occurred in circumstances of familial involvement, with some of her strains showing a cancerous incidence as high as 44 per cent. Lathrop and Loeb in 1915 found that the tendency toward tumor development in mice was associated with other recognizable hereditary characteristics, such as color, and that in addition to the element of appearance of spontaneous tumors there was another, entirely independent one—that of the age at which these tumors were most likely to appear. This observation has been recently confirmed by Turdeen. In crossed strains the results were not predictable, as some hybrids showed incidence rates corresponding to the higher parental rate, some an intermediate rate, and some the lower parental rate of incidence. In 1916 Slye published results that indicated clearly the homeotopic character of much of the hereditary tendency toward tumor development, as tumors of specific organs, and further of certain types, occurred repeatedly in certain strains, although rarely otherwise—particularly with reference to a strain of mice with an extraordinarily frequent development of the otherwise rare primary carcinoma of the liver; in 1919 she published similar data pertaining to primary tumors of the testis, of which she had seen, among 19,999 mice which had died of natural causes, 28 cases, all in 1 strain of mice or its hybrids.

While it may be regarded as firmly established that heredity, as studied in lower animals, plays a large part in the incidence of spontaneous tumors, the exact character of the hereditary element is still a matter of dispute. Loeb in 1920 expressed the belief that susceptibility to spontaneous cancer is, like that of inoculability with transplanted tumors, of mendelian type, but explicable only by the assumption of multiple factors. In 1922 Slye published results that would show that cancerous and noncancerous tendencies were transmitted as unit characters, which could be isolated in unit strains, and in 1926 she published additional data tending to show that tumor heredity was of mendelian character, with resistance as the dominant factor, with the tendency toward spontaneous tumors, both as to type and location, behaving like mendelian recessives. In 1928 appeared a report by her on a stock of Japanese waltzing mice which showed an incidence of thyroid carcinomas that she regarded as in strict accordance with the laws governing

mendelian recessives. Lynch, working with spontaneous and induced tumors of the lungs in mice, in 1926 reported that the spontaneous tumors showed a definite hereditary tendency with some indications that this was of dominant character, and in a report a year later on the development of tumors of the lungs in tarred mice, he found that the tumors in these circumstances appeared with relations that again fitted in with the action of a dominant factor. In 1928 Little criticized Slye's interpretation of her results in the belief that the incidence of tumors in her hybrid animals was too high to accord with the conception of mendelian recessivism. He suggested that the tendency to mammary cancer might be explained as a sex-limited mendelian dominant; the fact that Slye's results were too low to fit this concept without adjustment he met by the assumption that in homozygous forms this factor might be of lethal character. Some color is lent to this view by the observation of Stark of the occurrence of epithelial tumors in *Drosophila*, which, in addition to representing an instance of the rarely observed invertebrate tumors, were of sex-linked hereditary character, and were lethal in the larval stage.

As the occurrence of tumors of certain organs has been quite definitely associated with functional relations in others, there would appear to be evidence that factors other than the simple mendelian characters advocated by Slye may play a part in their hereditary causation. Loeb in 1923 found that in mice ovarian function was distinctly related to the incidence of cancer of the breast, and that the advent of this tumor could be almost wholly averted by castration early in life. Obviously such a relation would imply the action of independent factors, any or all of which might have hereditary relationships, and so would complicate the rôle of heredity into a complex almost impossible of unravelment. That it was necessary to assume the existence of a multiplicity of factors in the explanation of the incidence of human cancer on hereditary grounds was the belief expressed in 1923 by Aebly, and again in 1928 by Warthin, who considered that in man there are at least four intrinsic factors, any one of which might be recessive or dominant. Further, somatic variations of nontransmissible character may determine the incidence of tumors, as has been observed in mice by Strong.

The relations of heredity to the occurrence of tumors are further complicated by the undoubted influence of chronic or other irritation in the causation of cancer. The probable interrelations between irritative and hereditary factors were summarized in 1923 by Loeb as follows: With  $H$  representing the element of heredity,  $S$  a cancerogenic stimulus and  $C$  the cancerous state itself,  $H$  plus  $S = C$ . Obviously, either element of the left side of the equation could conceivably, and apparently actually, reach the necessary intensity alone. Numerous instances may be found in the literature of experimental cancers that



appear to bear out this conception. Fibiger in 1916 found that different, but rather closely related, rodents reacted differently in regard to the causation of cancer by *Spiroptera*, which excited cancer readily in piebald rats and only occasionally in white mice, even though the precursory lesions in the two animals were similar. Secher in 1920 found similar results in regard to oat-hair cancer, to which black rats were more resistant than other varieties. In regard to skin cancers caused by tarring, rats are notoriously almost absolutely immune, while mice are the most susceptible of the animals yet studied. With cysticercus sarcoma in rats, Curtis and Bullock found that there are marked familial differences in susceptibility. Slye observed in certain strains of her mice a marked tendency to the development of cancers at sites of local injury.

It may be questioned why, in view of the clear evidence of the importance of heredity in the incidence of spontaneous tumors in animals, there is so little indication of such relationship in the incidence of human cancers. A number of elements unquestionably contribute to the apparently largely negative character of human statistics. Even disregarding that emphasized by Wells—that of inaccuracy in the reports of human deaths<sup>1</sup>—it must be acknowledged that human data seldom extend over a sufficient number of generations to give an adequate conception of ancestral conditions. But even more important would appear to be the element stressed by Slye, the heterozygous character of human inheritance. Anything approaching the pure strains achieved by selective breeding of lower animals is of course impossible with man; and with the continuous and more or less enforced crossbreeding that has been exercised from time out of mind by human beings, it is only in exceptional circumstances that what there is reason to believe is an intricate hereditary effect can manifest itself openly as a distinctly familial incidence of cancer. But that such instances do occur the numerous cases cited in the literature show definitely.

#### CONDITIONS OF CANCEROUS GROWTH

In the development of a malignant tumor, there would appear to be a distinct possibility that two separable elements are concerned—first, the assumption by certain body cells of the property of unrestricted, malignant growth, and second, the existence within the body of conditions that permit their growth and survival. Although this second

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1. In connection with these views of Wells, it is interesting to note that de Bovis, who in 1910 studied the statistical evidence of heredity in its relations to human cancer, found that while the data of Germany and Holland indicated that that might be a factor, this was not shown by Hungarian statistics—a difference which he ascribed solely to the unequal diagnostic accuracy in the respective countries.

element is purely a matter of surmise, it is a conjecture that essentially lies behind almost all attempts to cure cancer by other means than that of direct extirpation or other methods of direct destruction of the tumor.

To what extent, if any, does the knowledge of cancer gleaned from clinical or experimental evidence throw light on the actuality or the nature of this second element? Clinical evidence throws little, except the occasional observation that cancers progress more rapidly in relatively young and well nourished persons. As concerns the experimental study of cancer, such knowledge as has been afforded by it is derived mainly from observations on transplantable tumors, and its significance as applied to tumors of spontaneous development is of necessity open to some question.

*Endocrine and Other Glandular Relations.*—Sex Glands: Among the earlier attempts to influence the progress of malignant disease by the induction of a more or less general alteration of metabolism was the procedure, suggested and practiced by Beatson, of oöphorectomy in cases of mammary cancer, with or without thyroid medication. In a number of patients treated in this way Beatson reported amelioration or temporary regression of the carcinoma, but the effect does not appear to have been permanent. Similar results were reported by other, principally British, surgeons, as by Lett, who in 1905 stated that some 36 per cent of patients treated in this way showed improvement, and as late as 1909 Cahen reported favorable results after castration in younger patients with cancer of the breast. Numerous studies have been made on the effect of this operation on the progress of implanted tumors in animals, with results that on the whole must be regarded as conflicting or indecisive. In 1909 Graff reported that castration was without apparent effect on the transplantability of tumors. Goldzieher and Rosenthal in 1912, and Hilario in 1915, likewise failed to observe any effect of this operation with the transferable tumors of rats and mice, and Engel in 1922 found little evidence of action on the growth of transplanted tumors by the sex organs. Joannovicz in 1916 stated that while with transplanted sarcoma in mice castration was without apparent effect, with carcinoma there was slight, on the whole rather indecisive, evidence of restriction of growth. Almagia, Loeper, and Turpin and Zizine, likewise observed limitation of the growth of implanted mouse carcinoma in castrated animals. Findings with the same general tenor of growth restriction have been reported by Strong, who found that mice castrated before maturity become entirely resistant to the inoculation of sarcoma, and as to the influence of the ovary, Fornero found that in mice transplanted tumors grew more rapidly and with less necrosis if the mice had been treated by injection of the follicular fluid of non-pregnant mice, while the reverse effect was obtained by the injection of the follicular fluid of pregnancy. Murphy and Sturm also observed

that in mice of both sexes castration during the first 7 weeks of life led to increased resistance to tumor implantation, and that there was some, though less evident, increased resistance even when the operation was performed early in adult life.

Results diametrically opposed to these have been observed by Sweet, Corson-White and Saxon, who reported in 1913 that castration of male mice accelerated the progress of transplanted tumors and increased the probability of successful implantation. Korentschewsky in 1914 and 1920 published accounts of castration experiments on a number of animals; with a transplantable round cell sarcoma of the dog, there was an acceleration of tumor growth, and the reverse effect of retardation was observed in animals treated by injection of fresh testicular extract; with a rat sarcoma similar effects were observed, while castration alone had no apparent effect on the progress of implanted mouse carcinoma. As regards the female sex organ, he observed some inhibition of growth after the injection of corpus luteum or ovarian interstitial tissue. Goldzieher and Rosenthal, and Engel, in similar injection experiments, observed little effect after the administration of ovarian or testicular extracts, and Elsner saw little change with the latter. Other experiments in which enhanced growth was observed after castration were those of Asada on transplanted tumors in mice.

**Adrenal Gland:** Similarly uncertain results have followed interference with other endocrine glands. As regards the adrenal gland, Joannovicz found that resection of that gland increased the percentage of "takes" of carcinoma in mice, but reduced that of the "takes" of sarcoma. Auler, working with a rat sarcoma originally produced by inoculation of *Bacillus tumefaciens*, found that after unilateral adrenalectomy there was a period of enhancement of growth, followed by marked degeneration and necrosis, at times to complete recovery and at times with regression of the primary tumor, but with acceleration of metastases. Pearce and Van Allen found inhibition of the growth of a transplanted rabbit carcinoma after extensive, though incomplete, destruction of adrenal tissue, and Flörcken observed that delayed growth followed bilateral adrenalectomy in the mouse; unilateral excision had little effect. Roffo reported that adrenal decapsulation greatly inhibits tumor growth. With injected adrenal extracts, Goldzieher and Rosenthal failed to observe any effect on the progress of mouse tumors. Arloing, Jusserand and Charachon found that after the injection of extract of the adrenal of a rabbit that had been immunized to the tumor used in their experiments, there was some restriction of the growth of mouse cancer—a result that Woglom was unable to confirm. Sokoloff reported that the injection of adrenal extract and iron into transplanted tumors of rats and mice caused their regression. Recently Coffey and Humber advocated the treatment of patients with extracts

of adrenal cortex on the basis of apparently favorable results. Sugiura and Benedict, Itami, Sigemitsu and McDonald, and Bischoff and Maxwell, with animal tumors, have been unable to obtain results in any way confirmatory. Auler and Rubinow, however, with implanted tumors, have observed marked regression after the injection of a number of preparations of adrenal cortex. They would explain this by an alleged effect of the promotion of polysaccharide metabolism, perversion of which Auler considered as an important element of cancerous predisposition. Flack attempted to solve the problem of the possible influence of adrenal secretion on tumor growth by implanting inoculable sarcoma either into the adrenal or along with adrenal tissue into skeletal muscle. No effect was evident with the tumors implanted in the adrenal itself, but with the double implants there resulted inhibition, complete or partial, of growth of the tumor, an effect that was lacking in control experiments in which spleen, liver, thymus, corpus luteum or testis was substituted for the adrenal tissue.

**Thyroid Gland:** Stimulation of tumor growth after extirpation of the thyroid gland was reported by Korentschewsky in the case of a dog tumor, and by Van Allen and Pearce with their rabbit carcinoma. With mouse tumors Korentschewsky observed the opposite effect, though to an insignificant degree. Engel found evidence of a restriction of tumor growth by the thyroid gland; Honda reported that the administration of thyroid retarded the progress of rat sarcoma. No effect from the administration of various thyroid products could be detected by Elsner, Uhlenhuth and Woglom, nor by Goldzieher and Rosenthal from the use of thyrocin. Flack, using his method of implanting tumor tissue directly into the organ, or along with it in some remote site, found with rat sarcoma that the thyroid-parathyroid system would stimulate tumor growth, the effect being somewhat dependent on proximity. With extirpation of the gland there was delayed growth of the tumor tissue. In tissue cultures, Zakrewski observed that the presence of thyroid stimulated the growth of the Jensen sarcoma—an effect that was likewise manifested, though to a less degree, by the addition of sex and salivary glandular tissue, all from young animals, and by rat embryonic tissue. As regards the parathyroid alone, Goldzieher found that injection of the hormone of that organ showed very slight evidence of stimulation of the growth of mouse carcinoma.

**Pituitary Gland:** In the case of the pituitary gland, Robertson and Burnett in 1916 studied the action on tumor growth of various extracts from the anterior lobe. Among the substances extractable by alcohol, tethelin and entire extract were found to increase the growth of transplanted tumor, while lecithin caused retardation. Engel found evidence of stimulation by this gland, and Elsner got variable results depending on the preparation used. Seel found that in rabbits the

injection of pituitary extract had no effect on developed tar cancers, although it appeared to delay their onset. In these experiments, however, there was a complicating factor of marked nutritional disturbance. Morphologic studies of the changes in the pituitary gland that occur in malignant disease have been made by Rohdenburg and Bullock and by Karlefors. The former found none of specific character; Karlefors reported in cancerous individuals a reduction in the number of the eosinophils and an increase in that of the chief cells, but these changes were not specific and were apparently secondary. The negative findings of Rohdenburg and Bullock apply to the results of a general study of the ductless glands in cancer, as made by them.

**Thymus Gland:** Pearce and Van Allen found some evidence that removal of the thymus gland was followed by stimulation of implanted rabbit carcinoma, and Engel also was of the opinion that it exerted a growth-restraining effect. Korentschewsky reported that feeding of thymic tissue hindered the development of implanted mouse carcinoma. Hanson reported that the administration of thymus to patients with cancer was followed by amelioration. On the other hand, Bullock and Rohdenburg and Johnson could detect no effect by this organ on tumor growth, in experiments consisting of its excision in rats implanted with the Flexner-Jobling rat carcinoma, and Elsner saw no results from the injection of thymus extract into mice that had tumors. Magnini found that after removal of the organ there was restrained growth of a rat sarcoma, and Mischtschenko, that thymus tissue, with that of the spleen and the adrenal, promoted the earlier stages of the growth of implanted tumors.

**Parotid Gland:** An isolated observation by Grünbaum and Grünbaum to the effect that the parotid gland influences tumor growth, inasmuch as they observed in growing tumors marked degenerative changes after its excision, could not be confirmed by Levin and Sittenfield.

**Lymphoid Tissue:** The relations of the lymphoid and reticulo-endothelial systems to cancerous growth have been the subject of repeated and extensive investigation. That lymphocytes play a large part in the so-called immunity to tumors was first suggested by the work of Wade on the infectious sarcomas of dogs, and for rat and mouse tumors by DaFano. Baeslack and Loeb and Harter also noted the importance of these cells in tumor defense, but that the action is one of protection against foreign tissue rather than against tumor as such was first indicated by the latter writers, who also recognized that this defense was not the sole one—a view that has been substantiated by the work of Mottram, Russ, and Russ, Chambers and Scott. Murphy and his co-workers, Hussey, Lieu, Maisin, Morton, Nakahara, Sturm and Taylor, in a series of experiments and reports principally on the effects of the x-rays on immunity to tumor, showed with some conclusiveness the important part taken by these cells in preventing

not only the growth of transferred tumors, but in general that of introduced foreign tissues. However, a number of more or less contradictory facts make the exact relationship of the lymphocyte to this defensive mechanism a matter of some uncertainty: The occurrence of lymphocytic accumulations within actively growing tumors, as found by Abetti, Woglom, and Loeper and Turpin; the recession of tumors without lymphocytic infiltration, observed by Bullock and Rohdenburg and, in the absence of lymphadenoid changes, by Woglom and Itami; the coexistence of tumor growth and a high blood content of lymphocytes, observed in the human being in lymphatic leukemia by Wood and in animals by Maeda and Wells; the growth of foreign tumors in close association with implanted splenic tissue in chick embryos, reported by Stevenson and Danchakoff—all indicate the complicated character of the relationships. The relation, however, would appear to be more particularly one of immunity to foreign tissue rather than to tumor growth, and its further discussion is not particularly pertinent here.

**Spleen:** Based largely on the fact that the spleen appears to possess a high immunity to the metastasis of malignant tumors, much experimental work has been devoted to the investigation of a possible rôle of this organ in tumor defense. In spite of the relative rarity with which metastases are found in the spleen, its freedom from involvement by tumor has been questioned particularly by von Hanseemann, who reported a number of instances of its involvement. Kettle suggested that its relative immunity may be explained on purely mechanical grounds, as due principally to its contractile power.

It was shown rather early that the injection of splenic tissue, even from the same animal, could prevent the successful subsequent implantation of transplantable tumor (Woglom, 1910); but that this effect would appear to be one of rather general immunity to tissue transfer is indicated by the work of Rohdenburg, Bullock and Johnson, and Woglom himself, among others, who found that this effect is not peculiar to the spleen, but is shared by a number of other tissues and organs.

Implantation of tumor tissue into the spleen has yielded negative results, the implants growing there about as freely as at any other site, as found by Fränkl, Goldman, Levin, Oshima, and Roffo and Encina, although some evidence of resistance has been noted by Cimoroni, Brancati and Lazarus-Barlow and Parry. The simultaneous insertion of spleen and tumor, on the other hand, has appeared to yield some evidence of an immunity effect, as restriction of growth has been observed in these circumstances by Fränkl, Biach and Weltmann, Mottram and Russ, Donati and d'Agata, with a variety of animal tumors. Fränkl's results, however, would scarcely indicate any specificity for this effect, since he obtained similar results when the tumor

tissue was mixed with blood or with hepatic or renal tissue. The simultaneous but separate insertion of tumor and spleen, used as a control by a number of the experimenters cited, failed to show the inhibitory effect. With similar experiments, Oshima was not able to detect any evidence of restraint of growth by splenic tissue. Fischer and Lumsden studied the effects of splenic tissue on cultured tumor cells, with negative results.

Studies of tumor growth in splenectomized animals, as pointed out by Woglom, are complicated by the factor of the possibility of replacement of splenic function by other lymphadenoid tissues. Oser and Pribram believed that splenectomy increased the growth rate of transplanted tumors, and that the injection of splenic tissue had the opposite effect, and Lewin also observed what appeared to be occasional curative effects from the injection of splenic material derived from other tumor animals, to the greatest degree when the implantation had been intraperitoneal. Results in general similar to those of Oser and Pribram have been reported by Korentschewsky and were obtained also, though less constantly, by Joannovicz, who observed increased tumor growth after splenectomy in 2 animals, but the reverse in 1. Serafini, who damaged the spleen by vascular ligation, believed that in this condition there was accelerated tumor growth, but Simpson, after damage to the organ by severe exposure to the x-rays, could detect no direct relations between splenic damage and susceptibility to tumor. Mottram and Russ were unable to detect any effect from splenectomy on the course of implanted tumors in otherwise normal rats, but with immunized animals there were found microscopic evidences of a proliferation of the tumor cells, too slight to be grossly evident. Perrachia also observed what he believed to be evidence of antiplastic activity on the part of the spleen, and Brüda reported that tumor tissues grow *in vitro* more intensively when the plasma used is taken from splenectomized animals. Bauer found that injected extracts of spleen have the effect of raising the surface tension of the serum, and believed that the tumor-inhibiting action of the organ rests in this feature. As regards more particularly the effect of splenectomy, in contrast to the foregoing results that would indicate antiplastic power, a considerable list of workers—Bullock and Johnson, Bullock and Rohdenburg, Dobrovoskaia-Zavadskaia and Samssonow, Donati, Morris, Oshima, Pearce and Van Allen, Woglom and Zeitlin—have been quite unable to detect any effects on the growth of tumors from splenic extirpation.

The frequency of enlargement of the spleen in tumor-bearing animals, especially in rats and mice, has been cited as evidence of specific relations between the organ and the tumor; but as Woglom points out in his general discussion of these phases of the cancer problem, this is too inconstant to be of evidential value, and is more

probably to be explained by concomitant or antecedent pathologic features, especially in the case of mice.

**Reticulo-Endothelial System:** The reticulo-endothelial system has been implicated more particularly in the causation of cancer by Erdmann, who observed that after blockage by injections of india ink it was possible to induce tumors of the Flexner-Jobling type in rats by means of apparently cell-free filtrates of that tumor; but some of her unprepared control animals also showed similar tumors, so that the exact effect of the blockage is questionable. Büngeler observed after similar blockage in mice the appearance of inoculation tumors after the injection of tumor cells damaged by chloroform beyond the point at which they would grow in more natural circumstances, and Lignac and Kreuzwendedich von dem Borne noted increased susceptibility to both mouse sarcoma and carcinoma in similar circumstances, as did Urban and Schnitzler. On the other hand, Theilhaber reported that in cases of human cancer improvement followed the blockage of the reticulo-endothelial system, and the isamine-blue therapy practiced by Roosen would appear to depend for its effect largely on the affinity of that material for reticulo-endothelial tissue. Psaromitas observed no effect from reticulo-endothelial blockage on the progress of transplantable tumor in the mouse, but in the fowl the Rous sarcoma was considerably restricted in its development by this procedure. Munck, working with mouse carcinoma, observed that there resulted from reticulo-endothelial blockage an inhibitory effect of transitory character, which disappeared after 16 days.

**Diet.**—Suggestions that dietary conditions might be in part responsible for an apparent increase in the incidence of human cancer are not infrequent in the earlier literature of that disease, although these seldom include any explanation of the manner of the supposed action. Williams, in 1896, was responsible for such a suggestion, and Sawyer in 1900, and both implied that the increased ingestion of meat was the responsible factor. In 1902 Williams, finding from statistical evidence that while cancer was relatively rare in the Jews of London, that people in the United States did not appear to enjoy a similar immunity, sought the explanation of this difference in the matter of dietary adequacy. McReddie, discussing in that year the occurrence of cancer in India from the same point of view, argued that its frequency there was not in evident contradiction to the alleged association of cancer with a meat dietary, since the population of that country is not as decidedly vegetarian as is usually assumed; but he was able to find no apparent relationship there between the prevalence of cancer and meat consumption. Needless to say, any great importance that may be attached to a flesh dietary largely vanishes when it is recognized that cancer is frequent in herbivorous



animals, and the interest now lies in the possible relationships of a general dietary to the occurrence of malignant disease—a possibility somewhat indirectly reflected in the article of Jacobson that appeared in 1907, in which he suggested that cancer might represent the result of diversion of excess energy from normal activities to unrestrained proliferation of tissue, and by the observations of Robertson and Ray that cancer is most likely to occur in mice with a relatively energetic growth rate, with a lead so obtained that usually persists for life.

**Protein:** The study of the behavior of the transmissible tumors of animals in relation to modifications of diet, even though accepted with the reservations that must attach to all study of tumors essentially foreign to their new host, affords some information in the line of present interest—the effects of modification of the individual on the progress of already established tumors. Indicative of the effects of general nutritional inadequacy on the growth of tumors are the experiments of Corson-White, Rous and Sugiura and Benedict, all of whom found that in the earlier stages an ample supply of food was essential to the progress of implanted tumors. As regards modification of the intake of protein, its results do not appear to be very considerable. Sweet, Corson-White and Saxon found that with a diet in which the protein elements were entirely of vegetable origin, the percentage of successful inoculations of tumor in mice was reduced to about one sixth of the normal proportion, and in a later article they believed that they could vary the susceptibility of animals by dietary alterations that affected thyroid activity, anything which tended to stimulate that organ increasing the probability of successful implantation, while thyroid-depressant substances decreased the number of “takes.” Rous, using the same modifications as those of Sweet, found that while with some tumors the restraining action was manifest even with tumors of considerable size, with others the effect was evident only if the under-feeding was begun before the growth of the tumors was well established. Some influence was exerted even on spontaneous tumors, to the extent that the restricted dietary would usually delay their recurrence after partial excision, as well as the appearance of metastases, a restraint that persisted only during the period of dietary restriction. That the effects of protein modification were more probably those of insufficiency rather than character appears from the work of Marsh, who found that mice on a vegetable diet containing an unusually wide range of protein substances were not affected as regards their susceptibility to implantation, and by Sugiura and Benedict, who found that the character of the protein material in the diet of rats and mice did not appreciably affect the growth rate of transplanted tumors, provided that such material was present to above 8 per cent of the food intake. Drummond found that while a diet low in protein would cause some retardation of

tumor growth in rats, this in general was associated with more general evidence of malnutrition in the form of severe loss of body weight. Apparently the requirements as pertains to amino-acids are just about the same as for general bodily welfare, since restriction of tumor growth occurred with deficiency of some of these, especially tryptophan, and restriction of the diamino-acids to the point of general inadequacy likewise retarded tumor growth.

**Fats and Lipoids:** As regards fat and lipoids, Robertson and Burnett, studying the effect of a milk diet on transplanted tumors in rats, found that with these animals there was some reduction in percentage of "takes," an effect which they ascribed to deficiency in cholesterol, particularly as they found that the injection of that substance accelerated the growth of implanted tumors, while lecithin had the opposite effect. The latter effect Bennett could not confirm, but Robertson and Ray, in a later paper, confirmed the earlier findings, although they were not evident in the case of spontaneous tumors, possibly because of the considerably shorter life of the animals. Moravek observed a similar accelerative effect from cholesterol with sarcomas, but not with mouse carcinoma. He found a depressant action of lecithin, evident with carcinoma even when administered along with small amounts of cholesterol—an effect which he ascribed to alteration of cellular permeability. Rondoni reported the observation of excessive tumor growth after the administration of cholesterol, and the opposite effect with lecithin, and Corson-White found that a high cholesterol content in the diet of tumor-bearing rats favored the onset of metastases. Bernstein and Elias found that the addition of either cholesterol or lecithin to the diet of tumor-bearing animals enhanced the growth of the tumors. Addition of an abundant supply of fat to the diet would appear to increase tumor growth; Beebe found this effect on the addition of butter, and Akematsu with lanolin.

Entirely unrelated to the action of lipoidal material through nutrition or through a presumably more or less direct effect on the cancer cells themselves is the action of certain materials of this general class on so-called immunity to tumor. Nakahara found that the injection of oleic acid or other unsaturated fatty acids or their salts, if this precedes by about ten days the implantation of tumors, serves to protect the animals so treated from the tumors. This effect he found was indirect and achieved through its effect on the lymphocytic reaction, and it appears to be similar to that observed by Lewin and Brancati, of immunity produced by the injection of nucleic acid. With olive oil, Nakahara observed an almost directly reversed effect, namely, the obliteration of the immunity to implantation of tumor which had been produced by the injection of homologous blood; this effect of reversion of potential immunity he also ascribed to modification of the lymphocytic reaction.

Carbohydrate: Van Ness, Van Alstyne and Beebe found that a deficiency of the carbohydrate fraction of the diet of animals into which tumors had been implanted was of little effect, unless it was begun at least several weeks before the implantation, but in that case there was marked restriction of progress of the tumors. Benedict and Lewis likewise found the carbohydrate content of the diet of great importance in determining the progress of tumors—rat sarcoma—and found that the administration of phlorhizin to tumor-bearing animals caused rapid disintegration of the tumors. From the addition of lactose to the diets of rats and mice with implanted tumors, Woglom was unable to detect any effect on the progress of the tumors, but apparently the dietary was already adequate before the addition. Händel and Tadenuma found with rats that an unbalanced diet with excess of carbohydrate favored tumor growth, an effect that was slightly accentuated by the injection of insulin, but they were unable to detect any change after the injection of phlorhizin. Goldfeder likewise found that abundance of carbohydrate favored tumor growth; however, with what would appear to be a greatly unbalanced diet with great excess of carbohydrate and insufficiency of other elements—an exclusively banana diet—Sugiura and Benedict found great restriction of the growth of the Flexner-Jobling rat tumor. The general effect of the carbohydrates in forwarding the growth of tumors does not appear to be entirely a matter of facilitating the growth of established tumors, as Rondoni found that the parenteral introduction of dextrose accelerates the action of tar on rabbits during the precancerous stages.

Vitamins: Joannovicz, and Fränkl and Fürer in confirmation, found that diets with a general deficiency of vitamins led to marked restriction of tumor growth, as did Ludwig. Such an effect was not observed by Kretschmar nor by Thies, nor by Benedict and Rahe, although in the experiments of the latter observers the animals were given sufficient vitamin B in the form of yeast to preserve their health. As concerns the various vitamins specially, vitamin A is regarded by Burrows as more or less identical with his hypothetic growth-restraining ergusia. Caspari and Ottensooser found that restoration of vitamin A to a vitamin-free diet enhanced tumor growth, but only in the presence of an adequate supply of vitamin B. Erdmann, in studying the development of spontaneous tumors in circumstances of vitamin deficiency, found that this was more likely to occur with absence of this vitamin and abundance of vitamin B. Drummond was unable to observe any restriction of tumor growth in rats on a diet deficient in vitamin A. With vitamin B, a more definite rôle would appear to have been established. The experiments of Erdmann, which are corroborative of Burrow's identification of this vitamin with his growth-promoting substance, archusia, have just been referred to. Drummond had found that while implanted tumors would grow for a time in animals on a diet

free from this vitamin, the growth would cease with the exhaustion of the animal's store of it. Funk failed to detect much effect from variation of this in the diet of fowls with the Rous sarcoma, but Heymann and Gallinek found that its absence greatly reduced the incidence of successful implantation of tumor in the rat. Caspari and Ottensooser, in a comprehensive survey of the work along this line, both as conducted by themselves and others, concluded that of the vitamins, B is the single one absolutely essential to tumor growth, even though absence of others may be of influence indirectly, as in the case of vitamin A or in that of vitamin D, the addition of which to the diet they found could enhance the growth rate of implanted tumors. As concerns vitamin C, Centanni found that with an exceptionally transplantable tumor of mice, if the animals were placed on a diet with especial restriction of this along with nuclein and phosphorus compounds as long as 10 days before implantation, there was a complete abolition of "takes," and that with already established tumors there was arrest and complete absorption if the growth had not gone too far.

**Mineral Salts:** In the case of mineral salts, principal attention has been devoted to the relations between potassium and calcium compounds and tumor growth. Beebe found in 1904 that there was a disturbance in the normal proportions of these salts, with a relative excess of potassium over calcium, in the case of rapidly growing tumors. Goldzieher in 1912 reported that in tumor-bearing mice treated by injection of salts of potassium or of calcium, there was, in the case of the former, an increase of tumor growth to about 48 per cent above that in the control animals, and in the case of the latter a reduction of 37 per cent. Sugiura, Noyes and Falk found in 1921 that immersion of the Flexner-Jobling rat tumor in solutions of calcium salts greatly inhibited its future growth, an observation that was confirmed in the following year by Troisier and Wolf, who observed the reverse effect with the use of potassium salts, and again by Goldfeder in 1928. The restraining effect on tumor growth of magnesium or its salts, reported by Reding with developing tar cancers, could not be confirmed by Itami for transplanted tumors. As regards the tumor-promoting action of potassium salts, Langfeldt observed that diets deficient in this metal hinder to a great degree the progress of transplanted tumors.

**Gaseous Metabolism:** Efforts to interfere with the development of tumors by interference with the general gaseous metabolism of the body have been made principally by Fischer, Buch Anderson, Demuth and Laser, and by Fischer-Wasels. All of their experiments were designed to promote oxidative metabolism, with the idea of suppressing anoxybiotic cleavage of dextrose, which Warburg found to be the predominant feature of tumor metabolism. In the experiments reported by the former group of observers, mice with implanted tumors were

kept in an atmosphere of oxygen under increased tension. A certain degree of healing of the tumors was observed in these circumstances, best when to the effect of the altered gas relations there was added that of injection of copper and selenium salts. Fischer-Wasels reported that even more striking results have followed the use of mixed oxygen and carbon dioxide, again with the added effect of injected colloidal iron preparations, or with that of injected dextrose and insulin. He reported that cancerous patients with such treatment show not only evidence of a restraint of the growth of the tumors but general improvement, as well. To what extent these results are to be explained by the theory on which the experiments were based, to what extent they were due to more general metabolic relations, is obscured by the findings of Campbell and Cramer to the effect that implanted tumors in mice and rats showed greatly diminished rates of growth when the animals were kept over long intervals under diminished oxygen tension, and by Woglom's finding that the progress of implanted tumors was not appreciably affected by induced acidosis or alkalosis. That the growth of a tumor is sensitive to general conditions of the body has been indicated by Mandl and Singer, who found that toxins due to fatigue favored the growth of implanted tumors in mice, as well as by the relations shown between pregnancy and the progress of the tumor—relations that appear to be inconstant. Von Graff found that with certain exceptions the growth of transplanted tumors was slower during periods of pregnancy, a finding confirmed by Slye. Mischtschenko found in rats with implanted tumors a retardation of neoplastic growth during pregnancy, while during lactation the tumors showed accelerated progress. Krotkina, on the other hand, with tar tumors in mice, observed accelerated growth during pregnancy.

*Summary.*—The more significant observations of the relations between tumor growth and conditions of the host would appear to be outstandingly the dependence of the former, at least in its earlier stages, on a general adequate nutritive supply, with special reference to that of carbohydrate and probably of fatty or lipoidal material. Endocrine disturbance has not shown any very definite relationships to the progress of malignant tumors. Of the vitamins, aside from indirect effects that would appear to be associated with more general nutritive disturbance, vitamin B would appear to have direct relationships to tumor growth. As regards relations of cancerous growth to mineral elements, the evidence of the action of potassium salts in facilitating, and of calcium salts in restraining, such growth, appear to be firmly established.

# Correspondence

## THE INTERNATIONAL ASSOCIATION FOR GEOGRAPHIC PATHOLOGY

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*To the Editor.*—A note concerning the organization of this Association has already appeared in the ARCHIVES (9:711, 1930). During 1930 and 1931 the national committees in various countries cooperated in a survey on the geographic distribution, racial variations and anatomic, physiologic and etiologic features of hepatic cirrhosis. The results of these surveys, submitted by each national committee were assigned for review and summary under five subdivisions, as follows: (1) Pathologic Anatomy of the Liver in Hepatic Cirrhosis, de Josselin de Jong, Utrecht; (2) Pathologic Anatomy of Other Organs in Hepatic Cirrhosis, R. Roessle, Berlin; (3) Clinical Features and Manifestations, Noel Fiessinger, Paris; (4) Etiologic Factors and Experimental Production of Cirrhosis, W. E. Gye, London; (5) Disturbances of Metabolism in Cirrhosis, F. C. Mann and J. L. Bollman, Rochester, Minn.

The presentation and discussion of these reports constituted the program for the first conference of the Association, held at Geneva, Oct. 8 to 10, 1931. No member of the committee for the United States was able to attend the conference. The assembled representatives agreed that the form known as Laennec's cirrhosis should be understood to include not only the granular contracted liver, with ascites, splenic enlargement and circulatory disturbance, but also a form of enlarged liver the fundamental histology of which is not essentially different. This obviates the confusion existing between Hanot's cirrhosis and this hypertrophic form of Laennec's cirrhosis. They recognized the existence of various intermediate stages or degrees of cirrhosis. The evidence that copper is an important factor in cirrhosis in man was not deemed sufficiently convincing. On the other hand, alcohol was considered the one known important factor, whether acting alone or in combination with infectious agents as suggested by evidence presented by workers in the United States.

The organization of the association in permanent form was an important feature. The provisory committee, which had voluntarily conducted the activities of the association, was replaced by a permanent executive committee of five members, one of whom is to be designated from the United States. The second conference was set for the autumn of 1934. The place of meeting and the subject for the next survey will be determined by the executive committee and will be announced later. Unfinished results and discussions concerning cirrhosis were deferred for consideration at the next conference.

Membership in the Association is open to those interested in this branch of medical science. One may become a member on recommendation of a member of the national committee of his country and the payment of the annual fee of 5 francs (Swiss).

There is a sentiment, in which the members of the Committee for the United States share, that the method of the first survey was perhaps too statistical. It is the hope of many that, as the organization develops, its scope and activities may be somewhat modified and that it will enlist the interest and cooperation of eminent workers in other divisions of medical science as well.

The official transactions will be published by Kundig, Geneva. These will include the five reports as given and the by-laws of the association.

V. H. MOON, M.D.,  
Chairman of the Committee for the United States,  
Jefferson Medical College, Philadelphia.

## Notes and News

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**University News, Promotions, Resignations, Appointments, etc.**—Sydney C. Dalrymple has been appointed instructor in pathology in Tufts College Medical School, Boston.

Antonio Dionisi, professor of pathologic anatomy in the University of Rome and well known for his researches on the etiology of malaria, died suddenly while addressing a congress on rheumatism.

A. Murray Drennan, professor of pathology in Queen's University, Belfast, has been appointed professor of pathology in the University of Edinburgh.

**Coroner's Office in San Francisco Becomes Appointive.**—According to the new charter of San Francisco, which has just gone into effect, the office of coroner is taken out of the elective group and placed in the appointive group, the present incumbent of the office becoming a life time appointee on good behavior.

**Professorship of Legal Medicine in Harvard Medical School.**—George Burgess Magrath, medical examiner in Boston, has been appointed professor of legal medicine in the Harvard Medical School. The professorship was established by a gift by Mrs. Frances Glessner Lee, Littleton, N. H. This appears to be the first endowed professorship of its kind in this country.

**Award of Gerhard Medal.**—The Gerhard Medal of the Philadelphia Pathological Society has been awarded to Alfred N. Richards, professor of pharmacology in the University of Pennsylvania, for his experimental investigations of renal function.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

THE CARDIODYNAMIC EFFECTS OF ACUTE EXPERIMENTAL MITRAL STENOSIS.  
L. N. KATZ and M. L. SIEGEL, *Am. Heart J.* 6:672, 1931.

A ligature method was developed to produce stenosis of the mitral orifice with a minimum of artefacts due to traction and with no interference with the coronary supply to the ventricle. The cardiodynamic changes were studied in dogs by optical manometers recording on bromide paper. The importance of recognizing, minimizing and evaluating the effects of artefacts is emphasized. Experimental stenosis of the mitral orifice produced the following immediate changes: a variable amount of slowing of the heart rate in most cases; a marked abbreviation of the ejection and total systole time in both ventricles; an elevation in the pressure of the left auricle; an increase in the magnitude of the left auricular contraction; a decrease in the maximum pressure developed by the left ventricle; a variable change in the level of the initial pressure of the left ventricle (due to an unavoidable artefact); a fall in systolic, diastolic and pulse pressure in the aorta; a variable change in the pulmonary arterial and right ventricular pressures—the pressure sometimes fell, sometimes rose or remained unchanged; the occasional appearance of presystolic oscillations on the left ventricular pressure curve; a steeper gradient of pressure rise during diastasis in the left ventricular pressure curve. These changes are in part the direct result of the stenosis, causing impediment of flow to the left ventricle and “damming back” of fluid in the pulmonary circuit. In part, they depend on the decrease in coronary flow resulting from the fall in arterial blood pressure. Compensatory mechanisms soon tend to restore conditions toward normal. Evidence is given which suggests that these compensatory mechanisms include: an increase in the pressure head of the left auricle, an increase in the magnitude of auricular activity, a prolongation of the time for diastolic filling and an augmentation of the aspirating action of the left ventricle as evidenced by the steeper rise of the diastolic portion of its pressure curve. All these factors tend to overcome the impediment of the obstruction, thereby augmenting left ventricular filling and lessening the damming up of blood in the pulmonary circuit.

AUTHORS' SUMMARY.

THE EFFECTS OF CERTAIN SUBSTANCES ON THE CILIATED EPITHELIUM OF THE GUINEA PIG. D. R. A. WHARTON, *Am. J. Hyg.* 14:109, 1931.

Bacterial poisons, light and certain other factors were found to inhibit ciliary movement in the tracheal epithelium of the guinea-pig, whereas bacterial toxins and venom failed to have any demonstrable effect. The inhibitory factors of the bacterial cultures (poisons) are considered to be of protein origin. Observations of the effect of antigen on the ciliated epithelium of sensitized guinea-pigs and on ciliated epithelium treated with precipitating antisera failed to elicit any change in the action of the cilia which might be used as a criterion of hypersensitiveness or immunity. The cilia were inhibited in different periods of time by various sputums, and it is suggested that the sputum in certain respiratory conditions, as bronchitis, may affect the movement of cilia adversely. The examination at autopsy of preparations from various parts of the respiratory tract may give valuable information as to the condition of the ciliated epithelium. Because of the simplicity of the technic, a great deal more attention should be given to ciliary function and the factors affecting it, by the use of preparations of ciliated epithelium from warm-blooded animals. The results obtained are more logically applicable to conditions in man than those obtained by using molluscs, protozoa or even frogs, and the further study of the subject should broaden ideas on the defensive mechanisms of the respiratory tract.

AUTHOR'S SUMMARY.



PRIMARY HYPOCHROMIC ANEMIA (ERYTHRO-NORMOBLASTIC ANEMIA). WILLIAM DAMESHEK, *Am. J. M. Sc.* **182:520**, 1931.

Seven cases of anemia are described, in which, although the symptomatology was that of pernicious anemia, the hematologic findings were those of "secondary anemia." Specimens taken from the sternal bone marrow for biopsy in three cases disclosed marked hyperplasia due to crowding with erythroblasts and normoblasts. The relationship of this anemia to the megaloblastic hyperchromic anemia called "pernicious" is discussed in detail. The possibility that the cases described are instances of chlorosis is discussed and rejected.

EXTRACTS FROM AUTHOR'S SUMMARY.

IDIOPATHIC HYPOCHROMEMIA. EDWARD S. MILLS, *Am. J. M. Sc.* **182:554**, 1931.

Evidence for considering idiopathic hypochromic anemia or hypochromemia as a disease entity is presented, and twenty-three cases of the disease are reported. The disease is confined to the female and runs a very chronic course with symptoms common to any moderately severe anemia. The important clinical features are an absence or a great diminution of free hydrochloric acid in the gastric contents, an absence of etiologic factors, a hypochromic blood picture and a tendency to resist ordinary forms of therapy for anemia. Twenty-one of twenty-three patients have been treated successfully with a capsule containing ferrous carbonate and copper carbonate U. S. P. Many of these cases had proved refractory to the iron alone before the iron-copper therapy was begun.

AUTHOR'S SUMMARY.

REGENERATIVE POSSIBILITIES OF THE CENTRAL NERVOUS SYSTEM. R. W. GERARD and R. R. GRINKER, *Arch. Neurol. & Psychiat.* **26:469**, 1931.

In a series of experiments on new-born rats and on fetuses in utero, the spinal cords were transected by a single clean cut with a cataract knife, and subsequently studied histologically, the "clinical" course of the animals being carefully observed during the interim. Many experiments yielded negative or no results because the animals died or the spinal column became displaced, etc. Several rats operated on at birth, however, gave evidence of a gradual return of function. One animal, studied with special care, which showed evidence of a complete section at the time of operation, was entirely paralyzed and anesthetic below the lesion for two weeks, while in a subsequent three weeks the recovery of sensation and voluntary motion was practically complete. The spinal cord of this animal showed no evidence of a lesion. Several rats operated on in utero were born from five days to a week later with the sense of pain, voluntary motion or both present in the hind quarters. The spinal cords showed partial lesions or none at all. We cannot decide on the correct interpretation of these cases. The possibility of the growth of nerve fibers into and across a lesion after a complete or partial transection is definitely established. The immature spinal cord also possesses remarkable powers of physiologic reorganization which may lead to full return of function below an almost complete separation of the cord. A consideration of the evidence bearing on regeneration in the mammalian central nervous system from other sources as well as from our own results indicates that although some growth of nerve fibers from the cut end of axons may appear, return of function due to anatomic regeneration has not been proved.

AUTHORS' SUMMARY.

INJURY AND REPAIR WITHIN THE SYMPATHETIC NERVOUS SYSTEM. S. S. TOWER and C. P. RICHTER, *Arch. Neurol. & Psychiat.* **26:485**, 1931.

The preganglionic sympathetic nerve fibers were cut on the right side before entry into the stellate ganglion in a series of twenty-six adult cats. Until the completion of regeneration and for from two to six weeks thereafter, records were taken of the skin-resistance of the fore paws and of the galvanic skin response.

Observations on sweating were also made. The effects of operation were: the immediate elimination of the spontaneous waves in skin potential and of the galvanic skin response; an immediate increase in skin-resistance. The resistance of the skin reached a peak many times the maximum normal figure between the second and fifth days after operation, and thereafter fell slowly and with fluctuation. Between the third and seventh weeks, as skin-resistance again approached a normal figure, the action currents of the skin reappeared. This reappearance was taken as the time of reconstitution of the connection between the central nervous system and the periphery. It averaged twenty-eight days. Evidence of some form of function of the synapse is noted several weeks before this date. The observations on sweating made at the same time indicate that the galvanic skin response is a far more delicate test for the presence of sympathetic innervation than gross observation of the activity of the sweat glands.

AUTHORS' SUMMARY.

THE NEURO-ANATOMY IN RESPIRATORY FAILURE. KNOX H. FINLEY, Arch. Neurol. & Psychiat. 26:754, 1931.

A careful anatomic study of two cases of poliomyelitis in which respiratory troubles were the prominent features led to the conclusion that the respiratory center is not represented by a definite group of cells in the medulla, but that several levels of the central nervous system are involved. The neural mechanism of respiration is thus integrative. One such level is in the reticular formation of the brain, which in Finley's cases was destroyed by foci of softening.

GEORGE B. HASSIN.

ACUTE EXPERIMENTAL GLOMERULITIS FOLLOWING REPEATED INJECTIONS OF HAEMOLYTIC STREPTOCOCCI INTO THE RENAL ARTERY. F. D. W. LUKENS, Bull. Johns Hopkins Hosp. 49:312, 1931.

A diffuse glomerulitis and interstitial inflammatory reaction could be obtained in the kidney of the rabbit by the repeated injection, at weekly intervals, of killed hemolytic streptococci into the left renal artery. This reaction was not observed after the first or after the second injection, but occurred in all of three rabbits four days after the third injection. It is suggested that the reaction is analogous to the Arthus phenomenon, which occurs under these conditions within an organ.

AUTHOR'S SUMMARY.

HYPERVITAMINOSIS. E. J. KING and G. E. HALL, Canad. M. A. J. 25:535, 1931.

The administration of massive doses of viosterol to chickens produced a condition of anorexia, loss of weight, extreme emaciation and finally death. Hypercalcemia resulted, and on histologic examination heavy deposits of calcium were observed in the secretory tubules of the kidneys. Histologic examination of the femurs showed the matrix of the trabeculae to be normal, but suggested a low amount of calcareous deposit within it. The percentage of ash, calcium and phosphorus of the bones, however, was normal. The bone phosphatase appeared to be present in less than the normal amount. The daily administration of parathormone appeared to produce no ill effects in chickens, comparable to those produced by viosterol. Histologic examination of the femurs showed productive fibrosis of the bony trabeculae without evident deposition of lime salts in the hyperplastic tissue. The mineral constituents and the phosphatase of the bone were present in normal amount.

AUTHORS' SUMMARY.

THE PERMEABILITY OF THE CUTANEOUS VENULES AND ITS FUNCTIONAL SIGNIFICANCE. F. SMITH and P. ROUS, J. Exper. Med. 54:499, 1931.

The permeability of the venules of the skin of the mouse greatly exceeds that of the capillaries. A mounting gradient of permeability exists along the further

portion of the latter. The significance of these facts is discussed with relation to conditions in human skin. The cutaneous venules are differentiated for several functions besides those ordinarily attributed to them, and must be considered as specialized organs.

AUTHORS' SUMMARY.

THE BONE MARROW IN FASTED AND POLYNEURITIC PIGEONS. R. A. MOORE and O. W. BARLOW, *J. Exper. Med.* **54**:761, 1931.

The histologic changes of the bone marrow in pigeons deprived of food and in pigeons with rice disease are essentially the same. The histologic changes of the bone marrow in pure vitamin B deficiency consist of degeneration, edema and slight endothelial proliferation of the small vascular channels, but with active hemato-poiesis. The anemia of rice disease in pigeons is in large part a starvation anemia and is not directly related to vitamin B deficiency.

AUTHORS' SUMMARY.

THE EFFECTS OF UNILATERAL NEPHRECTOMY ON OPEN GLOMERULI AND URINE OUTPUT. R. A. MOORE and W. W. SUMMERVILLE, *J. Exper. Med.* **54**:767, 1931.

Renal shock with oliguria or anuria after a unilateral operation on the kidneys was not produced in seventeen rabbits. For a period of two hours after unilateral nephrectomy, the one kidney carried on a proportionate part of the work of the previous two kidneys, and there was no evidence of functional compensation. There was a general correlation between the open glomeruli and the output of urine.

AUTHORS' SUMMARY.

THE CHOLESTEROL FUNCTION OF THE GALLBLADDER. R. ELMAN and J. B. TAUSSIG, *J. Exper. Med.* **54**:775, 1931.

Cholesterol determinations of gallbladder and hepatic bile obtained from the same source reveal a greater concentration in the former, even after the inspissating effect of the gallbladder is allowed for. This evidence, together with that from other experiments, indicates that the gallbladder has the power to excrete cholesterol into its lumen. There is evidence also that infection may accelerate this excretion. An hypothesis is presented to explain the precipitation of cholesterol in the bile, and the bearing of these findings on the pathogenesis of cholesterol stones is briefly discussed.

AUTHORS' SUMMARY.

THE GONAD-STIMULATING SUBSTANCES OF THE ANTERIOR LOBE OF THE PITUITARY BODY AND OF PREGNANCY-URINE. ZONJA WALLEN-LAWRENCE and H. B. VAN DYKE, *J. Pharmacol. & Exper. Therap.* **43**:93, 1931.

The assay of gonad-stimulating principles (hebin) by the use of immature male and female rats is discussed. The method used is based on the weight of the seminal vesicles in the male, and the ovaries in the female. The preparation of a crude pituitary hebin from various sources is discussed. It is pointed out that while beef anterior lobes are a poor source for this substance, good yields can be obtained from sheep and pork pituitary bodies. Pituitary hebin, administered subcutaneously, can initiate estrus in the hypophysectomized rat. Associated with estrus, there occur follicular maturation and uterine changes. Crude pituitary hebin does not dialyze through collodion or parchment; it is heat stabile (99 C.). It may be extracted from pituitary bodies at both acid and alkaline  $p_H$ . Hebin from pregnancy urine does not dialyze through collodion or parchment; it deteriorates when boiled in aqueous solution. Urinary hebin stimulates the testis and ovary with equal facility; pituitary hebin, on the other hand, stimulates the ovary in a much smaller dose than that having a moderate effect on the testis.

AUTHORS' SUMMARY.

RAYNAUD'S DISEASE: WITH SPECIAL REFERENCE TO ARTERIOLAR DEFECTS AND TO SCLERODERMA. THOMAS LEWIS and E. M. LANDIS, *Heart* 15:329, 1931.

There is a form of diffuse scleroderma in which those portions of the skin that are exposed are most affected; sclerodactyly and discoloration of the fingers on exposure to cold are the rule. The vascular defect underlying discoloration of the fingers in this disease is of the same nature as that occurring in Raynaud's disease of the usual type. Evidence is collected, which shows that in cases of severe Raynaud's disease (with or without diffuse scleroderma) the vessels to the fingers are altered structurally. The circulatory manifestations of Raynaud's disease are due to a local vascular defect. In milder forms this defect is expressed as a susceptibility to enter a state of spasm; in the severer forms, spasm is reinforced by local structural change.

AUTHORS' SUMMARY.

EXPERIMENTAL DIFFUSE HEPATITIS. GUY ALBOT, *Ann. d'anat. path.* 8:437, 1931.

Inflammation provoked by toxic substances is associated with early, parenchymatous, cellular lesions. The hepatic lobule functions from the periphery toward the center in pathologic conditions as it does normally. In the course of diffuse experimental hepatitis, it is possible to distinguish an initial periportal stage, a stage of tubular hyperergy and of reticulosis and finally a stage of "asymmetry" ("asymetrie lesionelle," an expression coined by Noel Fiessinger to show that at this period the lesion is less schematic than in the early stages). The acute, subacute and chronic varieties of diffuse hepatitis depend on the toxic properties and the dose of the drug, on the mode of its administration and also on the resistance of the animal. Acute diffuse hepatitis shows an atrophic centrolobular degeneration, which resembles acute yellow atrophy. The processes of experimental acute hepatitis are invariably the same although provoked by different agents. They always go through the same initial stages, showing elementary inflammation of the hepatic lobule. There is but one type of a diffuse inflammatory process in the liver. Cases differ, however, by their evolution, showing multiple aspects from an acute atrophic hepatitis to a cirrhosis. Experimental data go hand in hand with the observations made on human beings.

B. M. FRIED.

EXPERIMENTAL VASCULAR SCLEROSIS IN THE KIDNEYS OF RABBITS. N. GOORMAGHTIGH, *Ann. d'anat. path.* 8:585, 1931.

Chronic arterial hypertension obtained through enervation of the carotid sinus and section of the aortic depressive nerves causes a progressive hyalinization of the glomeruli. This is marked about three and one-half months after operation in instances in which the arterial pressure is maintained above 15 mm. It is accompanied by a slight interstitial sclerosis, which is particularly marked in the neighborhood of the glomeruli, and also by slight atrophic changes in some segments of the uriniferous tubules. Experimental, chronic, arterial hypertension also results in hyperplastic and degenerative lesions of the renal arterioles. The vascular and the glomerular lesions of the kidney are secondary to the arterial hypertension. The kidney is sensitive to prolonged variations in the arterial pressure, and the glomerular tufts are more sensitive than the afferent arteriole. The author also observed that rabbits with vascular sclerosis of the kidney showed a hyperplasia of the parathyroids.

B. M. FRIED.

SOME EFFECTS OF ROENTGEN RADIATION ON DIVIDING CELLS IN TISSUE CULTURES. H. W. LOVE, *Arch. f. exper. Zellforsch.* 11:435, 1931.

The radiosensitivity of a cell is a function of its displacement from maturity. If the backward displacement in time of a cell from maturity, that is, the state of the cell just preceding the division process, is less than about 180 minutes, its

radiosensitivity is constant and independent of the displacement. If the backward displacement in time of a cell from maturity is greater than about 180 minutes, there is a decrease in its radiosensitivity. The reduction in the number of dividing cells in an irradiated tissue culture is due to an inhibition of some fraction of the cells that normally would have entered mitosis during the process of irradiation. The temporary increase in the mitotic count of an irradiated culture after four hours' incubation is due to the superimposition of a complete or almost complete recovery of temporarily inhibited cells on an increased survival (due to decreased radiosensitivity) in the cell groups displaced from maturity to the extent of about three hours at the commencement of the experiment.

AUTHOR'S CONCLUSIONS [WILHELM C. HUEPER].

BIOLOGIC EXPERIMENTS WITH OVARIAN THYROID MATERIAL. A. PLAUT, *Klin. Wchnschr.* **10**:1803, 1931.

Iodine containing material from ovarian thyroid tissue gave positive results in experiments with acetonitril on rats and mice, as well as in repeated feeding experiments on tapoles.

### Pathologic Anatomy

CORONARY THROMBOSIS IN AN INFANT AGED FOUR MONTHS. ROBERT EWART RAMSAY and R. M. CRUMRINE, *Am. J. Dis. Child.* **42**:107, 1931.

Autopsy on an infant, aged 4 months and 8 days, disclosed thrombosis of the descending branch of the left coronary artery. The original lesion of the coronary artery appeared to be of an infectious nature, and was probably a bacterial embolus. In a search of the literature on coronary thrombosis, no report of the occurrence of this condition in so young a child was found.

FROM AUTHORS' SUMMARY.

NIEMANN-PICK'S DISEASE (ESSENTIAL LIPOID HISTIOCYTOSIS). H. G. PONCHER, *Am. J. Dis. Child.* **42**: 77, 1931; B. WASCOWITZ, *Am. J. Dis. Child.* **42**:356, 1931.

Poncher describes a case in a Jewish boy, aged 18 months, and Wascowitz one in a Jewish girl, 7 months old, and both give the observations at autopsy. The association of amaurotic family idiocy (Tay-Sachs' disease) and Niemann-Pick's disease is discussed.

PAUL MERRELL.

AORTIC ANEURYSM RUPTURING INTO THE CONUS ARTERIOSUS OF THE RIGHT VENTRICLE. E. H. SCHWAB and C. B. SANDERS, *Am. J. M. Sc.* **182**:208, 1931.

A case of acquired aneurysm of the ascending aorta with rupture into the conus arteriosus of the right ventricle is reported. The resulting physical signs simulate closely those of congenital heart disease. The two previously reported cases are briefly reviewed.

AUTHORS' SUMMARY.

CEREBRAL ANEURYSM AND CORTICAL HERNIATION. J. J. KEEGAN and A. E. BENNETT, *Arch. Neurol. & Psychiat.* **26**:36, 1931.

Cerebral aneurysm should be suspected in cases of spontaneous subarachnoid hemorrhage, particularly in persons under 40 years of age. Anatomic peculiarities of the cerebral arteries and infectious emboli are significant in the etiology of cerebral aneurysm. Arteriosclerosis and syphilis rarely are the cause. Focal neurologic signs frequently indicate the location of the aneurysm after rupture, rarely before, unilateral oculomotor palsy being the commonest localizing sign in

this series. Ligation of the internal carotid artery on the side of the lesion is indicated if recurrent hemorrhage occurs. Cortical herniation into arachnoid granulations is of common occurrence in conditions with intracranial pressure. An unusual case of thrombosis of the motor cortex from herniation into large pacchionian bodies is reported.

AUTHORS' SUMMARY.

CEREBRAL BIRTH PALSY. LEON FREEDOM, *Arch. Neurol. & Psychiat.* **26**: 524, 1931.

Study of a feeble-minded girl, aged 19, with signs of pyramidal and extra-pyramidal nerve lesions and epileptic attacks in whom the course of the disease was progressive, revealed avascular areas in the parietal, occipital and temporal regions of the cortex. The involved portions of the cortex were either degenerated or showed proliferation of Hortega cells and new formation of capillaries. Many areas appeared normal. The smaller blood vessels showed endarteritis. In some areas of the temporal lobe, only a few cells in the outer cortical layers remained. Equally severe were the lesions in the corpus striatum, where the small ganglion cells were more involved than the large elements, and where avascular areas were also in evidence. The pallidum was even more involved, and some portions were entirely devoid of ganglion cells. Glia rosetts were present in the optic thalamus, while the frontal lobe and the cerebellum, except the nucleus dentatus, were practically intact. The process was mainly degenerative. The thyroid gland showed a decreased amount of the colloid substance and hyperplasia of the connective tissue, with a diffuse small round cell infiltration of the entire gland substance. Freedom sums up the changes as degenerative, involving mainly the cortex and much less the basal ganglions and the nucleus dentatus. Clinically it was a case of infantile cerebral palsy, but anatomically it could not be classified.

GEORGE B. HASSIN.

DUODENAL ATRESIA. F. BATINI, *Pathologica* **23**:232, 1931.

In an instance of congenital duodenal atresia, a discontinuity of the mucous membrane alone was the cause.

E. HAAM.

GAMNA'S AREAS IN SYPHILITIC SPLEEN. G. PATRASSI, *Pathologica* **23**:266, 1931.

Siderous splenogranulomas (Gamna's areas) were found in great numbers in the spleen of a 5 year old child with congenital syphilis, and in the spleen of a 61 year old woman with numerous sclerogummatous lesions.

E. HAAM.

MULTIPLE DIVERTICULA OF THE SMALL INTESTINE. G. GIANNOMI, *Pathologica* **23**:277, 1931.

Thirty-six diverticula of different sizes and shapes were found in a piece of small intestine, 96 cm. long. The author explains the etiology on the basis of senile atrophy of the muscularis.

E. HAAM.

RELATION OF LYMPHOCYTES, MONOCYTES AND HISTIOCYTES TO EACH OTHER. G. SEEMANN, *Beitr. z. path. Anat. u. z. allg. Path.* **85**:303, 1930.

For his contribution to the controversial problem of the interrelationships of lymphocytes, monocytes and histiocytes, Seemann used chiefly the rat, because of the relatively high percentage of monocytes in the blood of this animal. Mice, rabbits, guinea-pigs and material from slaughtered domestic animals were also used. The work was done in Aschoff's laboratory. For the differentiation of the cells under consideration, chief reliance was placed on the method of supravital staining by neutral red and janus green. Smears and sections were also stained by the eosin-azure II and the oxidase method. The criteria accepted for the differ-

entiation of the cell types in supravital stained preparations are those laid down by Sabin and her co-workers. Seemann proposes the name monocytoïd for the cell of the blood and peritoneal and other fluids that has a nucleus like that of the typical monocyte but, instead of the characteristic neutral red roset of the latter, several relatively large clumps of coarser and finer neutral red granules and vacuoles. It is this type of cell, according to Seemann, that Maximow and his pupils have interpreted as a hypertrophied lymphocyte, through which are unfolded the multiple potencies ascribed to the lymphocyte by Maximow. The monocytoïd cell, in the opinion of Seemann, is not a hypertrophied lymphocyte in transition to a monocyte, polyblast or other type of cell. It has no relationships to the lymphocyte, but has relationships to the monocyte and has the same origin as the latter. The monocytoïd and monocytic cells are derived from an ubiquitous indifferent mesenchyme cell, and constitute, with the lymphocyte and the granulocyte, a distinct third leukocytic form. Having left the blood stream, the monocytes and monocytoïd cells are transformed into histiocytes. The histiocytes of an inflammatory area therefore have a double origin, coming in part from emigrated monocytes and monocytoïd cells, and in part from slumbering tissue histiocytes. Fibrocytes and capillary endothelia cannot be transformed into histiocytes. The reticulo-endothelia of Aschoff are closely related to the histiocytes. In lymph nodes in which an aseptic inflammatory reaction was induced, in tissue cultures of lymphoid tissues and in incubated blood and peritoneal fluid, Seemann could detect only regressive changes in the lymphocytes, and he believes that the occurrence of progressive and developmental changes in these cells still remains to be established.

O. T. SCHULTZ.

UNMASKING FRAGMENTATIO MYOCARDII. O. TAMURA, *Centralbl. f. allg. Path. u. path. Anat.* **52**:1, 1931.

After studies of fresh press preparations and serial and ordinary sections, the author concludes that the picture called fragmentation of the myocardium is an artefact due to folds in the muscle tissue. These folds are caused by atonic muscle fibers, which appear in contrast to the intact fibers. The name "pliciformatio myocardii" is suggested in place of the older term.

GEORGE RUKSTINAT.

LINGUAL STRUMA AND HYPOTHYREOSIS. P. HEILMANN, *Centralbl. f. allg. Path. u. path. Anat.* **52**:129, 1931.

A walnut-sized lingual mass of thyroid gland tissue was found in a 39 year old woman who died of pulmonary embolism following hysterectomy. No evidence of a thyroid gland was found in the usual location, although parathyroid bodies were noted. The superior and inferior thyroid gland arteries had normal origins, and the disposition of the external carotid and thyrocervical trunks was normal. The lingual thyroid tissue had alterations characteristic of a nodose struma. The position of the tumor just back of the foramen cecum seemed to point to a lack of descensus of the thyroid gland, which then remained small and hypoplastic and eventually formed a tumor. An analogy is drawn between the probable course of events in this case and that observed in undescended testes by Erdheim. As concerns the other glands of internal secretion, no thymic tissue was found; there were: small cystic structures in the intermediate zone of the hypophysis, but no disturbances of the cell relationships elsewhere; small fibrous ovaries, and nodules of cortical tissue of the suprarenal glands.

GEORGE RUKSTINAT.

URETER BIFIDUS CAUDALIS. S. SALTYKOW, *Centralbl. f. allg. Path. u. path. Anat.* **52**:177, 1931.

This rare condition was found in a woman, aged 60, who died shortly after a radical operation for sarcoma of the uterus. The accessory ureteral mouths were seen on cystoscopic examination, but were difficult to find post mortem.

The anomalous ureters were confined to the wall of the urinary bladder; the right was 4 cm. long, the left 2 mm. long, and both opened about 0.5 cm. above the main ureters. The natural ureteral orifices were only 1.2 cm. apart.

GEORGE RUKSTINAT.

THE CUTANEOUS CHANGES CAUSED BY THALLIUM ACETATE. J. VON VÁSÁRHELYI, *Dermat. Wchnschr.* 92:649, 1931.

Histologic study during and after complete epilation in young white rats given thallium acetate showed a low grade inflammatory process with infiltration of the hair follicles by polymorphonuclear leukocytes. Chemical tests revealed thallium in the skin. The author concludes that epilation by means of thallium is due to its direct action on the skin and that its excretion by the cutaneous glands results in inflammation, leading to loss of hair.

LAWRENCE PARSONS.

FATAL PULMONARY EMBOLISM. F. KAZDA and W. STOEHR, *Deutsche Ztschr. f. Chir.* 231:187, 1931.

The article contains an analysis of 145 patients who had been operated on and 152 patients with internal diseases who showed at autopsy occlusion either of the trunk or of one principal branch of the pulmonary artery. These instances were observed in 29,132 autopsies. The material is divided, for comparison, into two groups, the first embracing the period during the Great War (1915 to 1918) and the second the time between 1922 and 1928.

*Postoperative Embolism.*—The number of postoperative fatal embolisms decreased during the war, increased considerably in the period from 1922 to 1927, and decreased again slightly in 1928. Most of the fatal postoperative embolisms occurred in the months of December and March. There were many more women than men who died from postoperative pulmonary embolism, especially in the period after the war. Most of the persons concerned were from 50 to 60 years old; no postoperative fatal embolism occurred in childhood.

The greatest danger of embolism seems to be between the sixth and ninth days after surgical intervention, but the condition can occur even two or three weeks after operation. In the period from 1922 to 1928, not only was the frequency of embolism but also the size of the thrombotic masses increased. As the source of the embolism, thrombi were found as a rule in the veins of the right leg. Abdominal, especially gynecological, operations carry the greatest danger of embolism. Only one case was observed following resection of a goiter. The most frequent concomitant conditions were organic heart disease and vascular disease as well as pathologic changes in the kidneys, pleura and bronchi, less often in the liver and the bile ducts, and, in more than one third of the cases, in the spleen. General adiposity was present in one sixth of the surgical cases.

*Embolism in Patients with Internal Diseases.*—The fatal pulmonary embolisms in patients with internal diseases also showed a definite increase during the postwar period. They were more frequent in April, October and November than in other months. There were three times as many women as men in this series, and the majority of the patients were older than 60 years. The original thrombi were found as a rule in the veins of the right leg. In one third of the material, the principal disease consisted in organic changes of the heart and blood vessels, less often in those of the central nervous system. Diseases of the respiratory and digestive tracts followed in frequency. As concomitant diseases, changes in the respiratory tract were observed most frequently (more than two thirds of the cases). In more than one half of the cases there were organic changes in the liver and bile ducts, and in 50 per cent of the cases there were organic diseases of the heart and blood vessels. In more than 50 per cent, pathologic conditions were noticed in the urinary tract. General adiposity was present in one eighth of the medical material.



*Etiologic Considerations.*—The decrease in frequency of postoperative fatal embolism during the war and the increase in fatal postoperative embolisms and in those in patients with internal diseases in the years after the war indicate that the change in the nutrition during these different periods may have been responsible. The relative frequency of embolisms in adipose persons seems to point to the same factor. As contributing causes are suspected the less strict indications for surgical intervention during recent years and the longer duration of life due to more effective treatment of patients with internal diseases. There does not seem to be any evidence that epidemics of influenza played a rôle. The prevalence of women, especially of those in the menopause, suggests that postclimacteric changes of the female organism favor the development of thrombosis.

The almost complete absence of embolism after operations for goiter and the significance of the menopause are in favor of the assumption that internal secretion plays an important rôle in the etiology of thrombosis. The frequent coincidence of adiposity and embolism and, on the other hand, the occurrence of embolism in families suggest a certain relationship between constitution and embolism.

C. A. HELLWIG.

APLASTIC ANEMIA. H. ROSCH and G. HOLLAND, *Folia haemat.* **44**:48, 1931.

The authors discriminate between cryptogenic or a "virtual aplastic anemia" (described in the German literature as "hemorrhagic aleukia") and anemia with an aplastic blood picture. In the first, the bone marrow is primarily involved, while in the second, although the marrow is more or less affected, the disturbance is rather functional. They report one example of the virtual type with an unknown etiology. The second case was one which was thought to result from hemorrhoidal bleeding, but which was traced to benzene poisoning. In the third case, an isolated intestinal tuberculosis complicated anemia with an aplastic blood picture.

B. M. FRIED.

LYMPHOGRANULOMATOSIS OF THE BONES. R. DRESSER, *Strahlentherapie* **41**:401, 1931.

Twenty cases of lymphogranulomatosis of the bones are described. About 10 per cent of all cases of lymphogranulomatosis show involvement of the bones. The changes produced resemble, on roentgenologic examination, those caused by cancerous lesions. They are usually destructive, but a productive variety also occurs. The spine, pelvic bones, skull and sternum are most frequently involved.

WILHELM C. HUEPER.

TISSUE CULTURE: ITS SIGNIFICANCE FOR PATHOLOGIC ANATOMY. G. HERZOG, *Verhandl. d. deutsch. path. Gesellsch.* **26**:9, 1931.

While Roux was the first to study the development of explanted organs of the chick embryo in vitro, the modern practical method of tissue culture is entirely the work of American investigators (Harrison, Carrel, Burrow, L. Loeb, M. and W. Lewis). The new method has furnished important data on the microscopic structure of cells. The mitochondria are regarded as normal constituents of the protoplasm, and there is no transformation of mitochondria into pigment granules, fat drops or vacuoles. The melanin is formed—as A. Fischer demonstrated in cultures of embryonal iris and retinal epithelium—in finest granules, independently of the nucleus or the mitochondria. The duration of a mitotic division averages in vitro about from twenty-five to thirty minutes. Liquefaction of the culture medium is observed in different degrees and under various conditions. It is especially marked in cultures of normal mucous cells and in those of most malignant tumor cells.

Fibroblasts can be obtained from almost all organs of the embryonal and the adult organism. They form not only from the fibroblasts proper, but also from

endothelial cells, reticulum cells, periosteum and perichondrium, embryonal connective tissue and heart muscle. In the cultures of fibroblasts, all transitions between cell adhesions and syncytial arrangements may be observed, a fact that does not disprove the doctrine of the cell as the biologic unit, since also in the syncytial formations of the tissue culture the single cell territories seem to be preserved.

The macrophages play the next most important rôle in cultures of mesenchymal tissue. They are derived from the so-called histiocytes (reticulo-endothelial cells of Aschoff), adventitial cells, large mononuclear wandering cells and large monocytes of the circulating blood. These cell forms survive *in vitro* only a certain time; some of them are transformed into fibroblasts. A transformation of fibroblasts into macrophages, on the other hand, can be demonstrated in pure cultures only under special conditions.

The growth of epithelial cells *in vitro* is not always, as generally believed, less pronounced than that of fibroblasts. All epithelial cells have as a rule a tendency to form membranes. The growth of the cell carpet is due not only to cell division, but also to a harmonic ameboid movement of the cells.

The problem of cell differentiation was studied by Herzog and Schopper on different mesenchymal organs of guinea-pigs. When the culture medium was poor in embryonal extract, the development of argyrophil and later of collagen fibers was noted. Abundant provision of embryonal extract, on the other hand, interfered with the differentiation.

In most tissue cultures, a large central area of the explanted tissue fragment undergoes necrobiosis. Only a peripheral zone survives, and from there the proliferating cells migrate into the culture medium. In cultures of the spleen or of the lymph glands, small lymphocytes and leukocytes migrate into the medium immediately, followed about fifteen hours later by the so-called macrophages, while the fibroblasts do not migrate before the second or third day.

The behavior of blood vessels was studied in tissue cultures of the omentum. In the first hours there is a migration of adventitial cells. While the endothelial elements first show proliferation, soon progressive dissolution of the capillaries takes place. From the second or third day on, the endothelial cells migrate into the medium, and large portions of the capillaries disappear. From artery stumps, straight, often long, buds grow out and unite into a network as in the living organism. In later stages, however, the endothelial cells proliferate diffusely and cannot be distinguished from cultures of fibroblasts. Schopper observed even the forming of argyrophil and collagen fibers in cultures of endothelial cells.

The spleen is the most frequently cultivated organ. There is wide dissension of opinion regarding the behavior of the lymphocytes. Most of those that migrate in the first hours die. The proliferating lymphocytes of the peripheral zone are larger and take vital stain. Not every small lymphocyte can change into a large macrophage. Also in cultures of lymph nodes, most of the small, immediately migrating lymphocytes die. Maximow, using lymph nodes of the rabbit, to which he added extract of bone marrow, demonstrated the development of granulocytes from ungranulated cells.

Carrel and Ebeling obtained pure cultures of monocytes from leukocytes, and Maximow traced the development of argyrophil fibers to blood cells. But Herzog is of the opinion that fibroblasts originate only from the so-called monocytes or histiocytes of Aschoff.

When tubercle bacilli are added to tissue cultures, a specific proliferation of epithelioid and giant cells is noted, which have phagocytic properties. In cultures of blood cells, tubercle bacilli cause the formation of epithelioid cells, a fact that suggests the possible hematogenous origin of epithelioid cells in the living organism. From the observation that in some tissue cultures bacilli containing fibroblasts are found, the inference may be drawn that the formation of collagen fibers in tubercles is due to the fibrillogenic potency of epithelioid cells.

The proliferation of epithelial cells *in vitro* resembles that in the body during regeneration or organization. Pure cultures of epithelial cells can be regarded

as permanent regenerative states. Often the culture of epithelial cells resembles the atypical proliferation in the body, but a transformation of normal epithelial cells into true carcinoma has never been accomplished in vitro.

The superficial cells of serous membranes grow first in epithelium-like fashion, forming continuous carpets, and liquefy the plasma, but in latter passages they proliferate like fibroblasts, and there is no liquefaction of the medium noted. Schopper demonstrated the development of argyrophil fibers between proliferating serosa cells, thus the epithelial and mesenchymal properties of serosa cells are well illustrated in tissue cultures.

From the morphologic point of view, the tumors of animals have been thoroughly investigated, while the cultivation of human new growths is still rudimentary.

Cancer cells grow in vitro, like normal epithelial cells, forming membranes. By adding fibroblasts, the formation of solid strands and tubules and cornification are favored. The time of mitotic division does not seem to differ in normal and malignant cells; the incidence of mitotic figures is, according to A. Fischer, much higher in cultures of carcinoma. The number of chromosomes is smaller in the cells of mouse carcinoma than in normal cells, and the form and size of the chromosomes vary considerably.

Herzog concludes from this review that growth and histogenesis in tissue culture follow the same principles as in the living organism.

C. A. HELLWIG.

## Microbiology and Parasitology

DISSOCIATION OF DIPHTHERIA BACILLUS. M. E. MAVER, *J. Infect. Dis.* **49**:9, 1931.

Variants of the diphtheria bacillus are described as they appeared in a study of the growth and the production of toxin of this bacillus in synthetic mediums. The dissociation seemed to progress in gradual stages to the more attenuated coccoid form. The yellow and pink pigmentation of pellicles and colonies in attenuated cultures was observed, as well as all variants described by others. The antigenic relationships between the variants and the original Park 8T strain indicate a closer relationship between the rods and the rodlike forms and the original strain than between the coccoid forms and the original strain. The fermentation reactions of the variants differed from those of the original strain in some cases. Of thirty-one freshly isolated strains of diphtheria bacilli grown on synthetic agar, ten showed dissociation into coccoids after the first transfer and fifteen after the second. The use of synthetic medium on which the diphtheria bacillus can become adapted to growth and production of toxin is recommended for the study of the dissociation of this micro-organism.

AUTHOR'S SUMMARY.

DISTRIBUTION OF DIPHTHERIA BACTERIOPHAGE. G. H. SMITH and ELIZABETH F. JORDAN, *Yale J. Biol. & Med.* **3**:423, 1931.

Phage for *Bacillus diphtheriae* has been obtained from sewage; from the throat washings, stools and urine of patients with diphtheria (in one instance ten days after the organism had disappeared from the throat); from carriers; from the throats of persons who were not carriers, or from whom the so-called diphtheroid forms could not be recovered by ordinary cultural methods; from the air and from the floor sweepings of laboratories in which work with diphtheria bacteriophage was being conducted, and from 100 per cent of all field cultures of diphtheria bacilli subjected to examination. The significance of this wide dissemination of diphtheria bacteriophage cannot be determined. Whether or not it represents an exceptionally delicate index of contamination pointing to a wider distribution of diphtheria bacilli or of forms allied to them remains unsolved. The relationship, if any exists

between diphtheria bacteriophage and diphtheritic infection, is likewise unknown. Possibly some of the conflicting reports of the spontaneous appearance of bacteriophage may be explained by the demonstration that the bacteriophage corpuscle or the lytic principle adherent to particulate matter may be recovered from the air and from dust.

CATAPHORESIS EXPERIMENTS WITH TYPHUS VIRUS. I. J. KLIGLER and L. OLITZKI, Brit. J. Exper. Path. **12**:69, 1931.

Experiments are reported bearing on the electric charge of the virus of typhus and the possibility of recovery of the virus by cataphoresis from tissues of immune animals. The results of the experiments show that the virus wanders to the positive pole. This may signify either that the virus carries a negative charge or that this is carried by the associated proteins. It has not been possible by cataphoresis to obtain the virus from tissues of animals that had recovered from typhus infection. It has not been possible to separate the virus from virus-antiserum mixtures by cataphoresis of suspensions of  $p_H$  6 and 8.6, respectively. So far as recovery of the virus from tissues of immune animals is concerned, the virus of typhus differs from the filtrable viruses studied by Olitsky and his associates.

AUTHORS' SUMMARY.

THE EFFECT OF THE X AND V GROWTH-FACTORS ON THE PATHOGENICITY OF INDOL-PRODUCING STRAINS OF INFLUENZA BACILLI. A. B. ROSIER, Brit. J. Exper. Path. **12**:133, 1931.

The experiments described indicate that the pathogenic action of a microbe that does not produce any notable quantity of toxic substance in vitro can be disclosed more readily by introducing with it its necessary growth factors. The healthy peritoneal cavity does not contain the necessary factors, and therefore influenza bacilli will not grow therein. If, however, these factors are supplied either by injury to the tissues as a result of the injection or by including them in the inoculum, growth can take place, and toxic substances may then be produced under conditions more favorable to their preservation than in a test tube. The application of this principle to the influenza bacillus shows that 71 per cent of indol-producing strains are pathogenic in mice, whereas those that produce no indol are practically nonpathogenic.

AUTHOR'S SUMMARY.

STUDIES ON PROTEIN-FREE SUSPENSIONS OF VIRUSES. I. J. KIGLER, L. OLITZKI and M. ASCHNER, Brit. J. Exper. Path. **12**:178, 1931.

Cataphoresis experiments with protein-containing and protein-free suspensions of a *Bacillus coli* phage and fowl-pox virus indicate that the results heretofore reported have been influenced by the protein. The charge measured was therefore chiefly that of the protein to which the virus was adsorbed. On the basis of the results in protein-free suspensions, it appears that both the *B. coli* phage and the fowl-pox virus are sensitive to acid reactions, and carry positive or negative charges according to the reactions of the medium. The phage is amphoteric in acid and decidedly alkaline solutions, and chiefly negatively charged in neutral and mildly alkaline solutions. The fowl-pox virus is positively charged on the acid side, is amphoteric in neutral solutions, and carries a negative charge in alkaline solutions.

AUTHORS' SUMMARY.

THREE CASES OF PSITTACOSIS WITH TWO DEATHS. H. R. FISHER and R. J. HELSBY, Brit. M. J. **1**:887, 1931.

Psittacosis is still liable to occur in this country, and should be borne in mind when one or more patients in a household are suffering from an influenzal type of illness, with early signs in the lungs and perhaps some typhoid-like symptoms,

such as epistaxis, abdominal distention, vomiting, constipation or diarrhea. Especially should such a diagnosis suggest itself if influenza is not prevalent in the neighborhood. Parrots are not the only birds that cause psittacosis in man. In the report of the Ministry of Health (Reports on Public Health and Medical Subjects, no. 61, London, H. M. Stationery Office) lovebirds, thrushes and canaries, among other birds, are stated to have been the cause of human illness. It is not certain how many types of birds suffer from diseases communicable to man, so the fact that a patient keeps birds should arouse suspicion in doubtful cases of human illness. All bird-keepers should take care always to wash their hands after attending to the birds, and especially before taking food. An unusual factor in the cases described in this article is that three generations of human psittacosis followed from the original sick budgerigar. Cosman, quoted in the Ministry's report, mentioned a woman who caught psittacosis from a patient whom she nursed and afterward infected her own child, but such a sequence is sufficiently unusual to be worth noting. The infection of a trained nurse in the course of her duties shows the care required in dealing with psittacosis, and incidentally, therefore, the necessity of early diagnosis of the disease. The nurses attending these patients were warned to treat them like patients with typhoid, and this is probably all that can be done. So far as is known, this is the first instance in this country of the infection of a hospital nurse with psittacosis by her patient, and, even so, two of the three nurses in attendance escaped the disease.

AUTHORS' SUMMARY.

MICROBIC DISSOCIATION OF BCG. R. S. BEGBIE, Edinburgh M. J. 38:174, 1931.

Dissociation of BCG into rough, smooth and intermediate colonial types is found. Although the evidence of virulence is not clearcut, the smooth type is the most virulent, the rough type intermediate in virulence and the intermediate type least virulent. The smooth type is described as moist and shining, with a central raised dome. The intermediate colony has an umbilicated center with an irregular, narrow fringe, which does not spread. The rough type is composed of heaped up coils without a fringe, and has a waxy appearance.

EDNA DELVES.

ACTION OF ACETONE EXTRACTS OF TUBERCLE BACILLI. B. IAKHNIS and S. CHAGALOVA, Ann. Inst. Pasteur 46:579, 1931.

Subcutaneous inoculations of acetone extractives of tubercle bacilli favored the development of lesions in guinea-pigs previously inoculated with the filtrable elements of tubercle bacilli. After six or seven injections, the site of inoculation of the filtrate usually showed caseous or noncaseous ganglions containing the organisms. The invasive capacity of isolated organisms seemed to be greater the longer the course of the injections of the extract and, correspondingly, the more marked were the caseous nodes from which the organisms were recovered. This invasive capacity seemed in several ways to correlate with the pathogenesis as influenced by acetone extract. The activity of the extract appeared specific. Diagnostic application is suggested, with the use of serial injections of extract in animals receiving pathologic material potentially containing the tuberculous "ultravirus."

M. S. MARSHALL.

THE DIAGNOSIS OF TUBERCULOSIS IN MONKEYS. A. NOILEN and M. SARVAN, Beitr. z. Klin. d. Tuberk. 77:186, 1931.

The usual tuberculin tests are unreliable in monkeys. Following the intramuscular injection of from 3 to 5 cc. of a mixture of ophthalmotuberculin and old tuberculin, tuberculous monkeys die within from one to three days. The same injection has no effect on nontuberculous monkeys.

MAX PINNER.

THE SIGNIFICANCE OF DOSE IN REACTION TO TUBERCLE BACILLI. E. SANTO, Beitr. z. Klin. d. Tuberk. **77**:191, 1931.

Different amounts of bacilli injected into the cornea produce different degrees of tissue reactions, but the quality of the histologic picture is always the same. The same observation was made on subcutaneous injections into guinea-pigs. The regional lymph nodes, however, show always, regardless of the amount of bacilli, maximal reactions. The quality of the tissue reaction, both locally and in the regional lymph nodes, was not effected by dosage. By counting the tubercle bacilli in foci of human pulmonary tuberculosis it was found that the quality of the histologic picture was independent of the number of bacilli.

MAX PINNER.

AGRANULOCYTOSIS. W. HUEBER, Frankfurt. Ztschr. f. Path. **40**:312, 1930.

Hueber believes that agranulocytosis is not a disease per se, but a complex of symptoms in certain cases of infectious diseases. The term "agranulocytic septicemia" seems more appropriate. The disease occurs mainly in women, but is also encountered in the other sex. It is not found more frequently during puberty or during the climacterium as was suggested by some authors. A generalized bacterial infection seems the important etiologic moment. Such infection might be detected either by the history of the case and the clinical course, or by a bacteriologic, anatomic or histologic examination. There are no grounds for the belief that a specific inflammation may give the clinical picture of agranulocytosis. Either increased virulence or chronic action of bacteria might be responsible for such a condition. It is probable that the toxins in such cases produce paralysis of the bone marrow.

O. SAPHIR.

SPIROCHETES IN THE EYE FOLLOWING SUBSCROTAL INOCULATION OF SYPHILIS. KEIHO KAMADA, Klin. Wchnschr. **10**:1116, 1931.

The corneal tissues in five of ten syphilitic rabbits contained spirochetes when examined at varying intervals after scrotal inoculation. Spirochetes were not found in the lens and vitreous chamber.

AUTHOR'S SUMMARY.

BACTERIAL COUNTS IN ARTERIAL AND VENOUS BLOOD IN ENDOCARDITIS LENTA. ERICH ZDANSKY, Ztschr. f. d. ges. exper. Med. **76**:571, 1931.

Bacterial counts were made in ten cases of endocarditis lenta, the blood being taken from the brachial artery and from a vein of the elbow. Of nineteen positive cultures, nine showed a distinctly higher count in blood taken from the artery, nine very little difference in count, and one a distinctly higher count in blood from the vein.

PEARL ZEEK.

FILTRABLE FORMS OF THE TUBERCLE BACILLUS. G. W. SCHMIDT, Ztschr. f. Hyg. u. Infektionskr. **112**:95, 1931.

A great number of experiments and a large number of guinea-pigs used in them gave no evidence for the existence of a particular pathogenic, filtrable, invisible form of the tubercle bacillus. The positive results of other authors are questioned. Old tuberculin from commercial and other sources contained large quantities of dead organisms, which were unaltered in form and acid-fast staining.

EDNA DELVES.

TUBERCULOSIS OF PLEURAL LYMPH NODES. R. ROOTS, Ztschr. f. Tuberk. **60**:125, 1931.

In 200 necropsies on patients who had not died of pulmonary tuberculosis, the pleural lymph nodes were examined. All patients were 35 years or older. In

46 of these, little nodules were found in or immediately below the pleura. In some cases, multiple nodules, a total of 147, were found. On microscopic examination, 36 of the 147 foci were not lymph nodes and did not show tuberculosis. One hundred of them were pleural lymph nodes. Seventeen of these lymph nodes showed insignificant changes, such as anthracosis and fibrosis. Eight of these lymph nodes showed hyaline nodular scars, which did not show anything characteristic of tuberculosis. But these nodes that came from 7 different patients are the only lesions that could possibly have been caused by tuberculosis. These findings are totally different from those reported by Anders and Schmöe. It is concluded that pleural lymph nodes are not infected hematogenously, but directly from the pulmonary tissue.

MAX PINNER.

#### METAL SALT THERAPY IN EXPERIMENTAL TUBERCULOSIS OF GUINEA-PIGS.

L. E. WALBUM, *Ztschr. f. Tuberk.* 60:204, 1931.

Guinea-pigs were infected with virulent bovine bacilli which killed controls within seventy-nine days with generalized tuberculosis. Twenty animals were treated, ten receiving cadmium chloride and ten manganese chloride. Treatment was started fifteen days after the infection, when the animals had palpable enlargement of the lymph nodes. The manganese salt was used in concentrations of from  $10^{-5}$  to  $10^{-10}$  molar, and the cadmium in a concentration of from  $10^{-7}$  to  $10^{-11}$  molar. About half of the guinea-pigs treated did not show any sign of tuberculosis at autopsy, which was never performed earlier than three months after the infection.

MAX PINNER.

#### ISOLATION OF TUBERCLE BACILLI FROM THE BLOOD OF TUBERCULOUS PATIENTS.

K. JONTOFSOHN, *Ztschr. f. Tuberk.* 61:35, 1931.

An attempt was made to isolate tubercle bacilli from the blood of forty-one patients with pulmonary tuberculosis and nine patients with surgical tuberculosis. Löwenstein's recent method was used. Only in three cases of pulmonary tuberculosis was it possible to demonstrate the presence of tubercle bacilli in the blood.

MAX PINNER.

#### PATHOLOGIC ANATOMY OF UNDULANT FEVER. F. GREGERSEN and T. M. LUND, *Hospitalstid.* 74:349, 1931.

In undulant fever there may be enlargement of the spleen, with congestion and hyperplasia of the pulp, but shrinking of the follicles; foci of granulation tissue in the spleen, liver and other organs; also parenchymatous degeneration.

### Immunology

#### IMMUNOLOGICAL STUDIES OF COLDS AND INFLUENZA. G. HOWARD BAILEY, JANET M. BOURN and V. A. VAN VOLKENBURGH, *Am. J. Hyg.* 14:453, 1931.

The case discussed presents the following interesting features: (1) a natural respiratory infection which, from bacteriologic and serologic evidence, was presumably a true primary infection with *Hemophilus influenzae*; (2) an illness which did not resemble true influenza in that it was afebrile, lacked prostration, requiring confinement in bed, and was comparatively mild in its effect; (3) leukocytosis during the acute stage of the illness; (4) cultures from the pharynx and tonsillar fossae that were positive for *H. influenzae* at least twelve weeks after the onset of the illness, prior and subsequent cultures having been consistently negative; (5) serologic evidence (by means of complement-fixation tests prior to and following the illness) of a definite increase in the antibody content of the blood against

homologous and heterologous strains of *H. influenzae*, evident three weeks after the onset of the illness, greatest at the end of seven weeks and approaching the level observed prior to the illness during the succeeding weeks; (6) as determined by the maximum titer, one homologous strain of *H. influenzae* (1522) that appeared to be the most specific in antibody response.

FROM AUTHORS' SUMMARY.

**STREPTOCOCCAL AGGLUTININS IN CHRONIC INFECTIOUS ARTHRITIS AND RHEUMATIC FEVER.** E. E. NICHOLLS and W. J. STAINSBY, *J. Clin. Investigation* **10**:323 and 337, 1931.

The serums of patients with chronic infectious arthritis usually give a strong specific agglutination with "typical strains" of streptococci recoverable from the blood and joints of patients with this disease. Control serums do not show this reaction.

Chronic infectious arthritis can be differentiated from degenerative arthritis and from chronic polyarthritis following rheumatic fever by the agglutination reactions. These suggest different etiologies for the three forms of arthritis.

A close antigenic relationship between "typical strain" streptococci and the hemolytic streptococci from scarlet fever and erysipelas is established.

Additional evidence is presented in support of the theory that streptococci of the nonhemolytic type are important etiologic agents in rheumatic fever. This evidence consists in the demonstration of streptococcal agglutinins in the serums of patients with rheumatic fever.

Chronic progressive polyarthritis following rheumatic fever, although presenting a clinical picture similar to that of primary chronic infectious arthritis, gives evidence by agglutination reactions of being etiologically different.

Further evidence is presented of the etiologic relationship between the rheumatic fever and subacute bacterial endocarditis.

AUTHORS' SUMMARIES.

**CULTIVATION OF VACCINE VIRUS FOR JENNERIAN PROPHYLAXIS IN MAN.** T. M. RIVERS, *J. Exper. Med.* **54**: 453, 1931.

A dermal strain of vaccine virus has been adapted to a simple culture medium consisting of minced chick embryo suspended in Tyrode's solution. The bacteria-free culture virus thus obtained produces in lower animals and in man typical vaccinia that renders them refractory to infection with ordinary vaccine virus harvested from calves.

AUTHOR'S SUMMARY.

**THE USE OF MICE IN TESTS OF IMMUNITY AGAINST YELLOW FEVER.** W. A. SAWYER and W. LLOYD, *J. Exper. Med.* **54**: 533, 1931.

A method of testing serum for protective power against yellow fever is described and designated as the intraperitoneal protection test in mice. The test consists essentially of the inoculation of mice intraperitoneally with yellow fever virus, fixed for mice, together with the serum to be tested, and the simultaneous injection of starch solution into the brain to localize the virus. If the serum lacks protective power, the mice die of yellow fever encephalitis. The test is highly sensitive. Consequently it is useful in epidemiologic studies to determine whether persons have ever had yellow fever and in tests to find whether vaccinated persons or animals have been immunized. When mice were given large intraperitoneal injections of yellow fever virus fixed for mice, the virus could be recovered from the blood for four days, although encephalitis did not occur. If the brain was mildly injured at the time of the intraperitoneal injection, the symptoms of yellow fever encephalitis appeared six days later, but the virus was then absent from the blood. Strains of white mice vary greatly in their susceptibility to yellow fever.

AUTHORS' SUMMARY.



CUTANEOUS REACTIONS IN RABBITS TO THE TYPE-SPECIFIC CAPSULAR POLYSACCHARIDES OF PNEUMOCOCCUS. T. FRANCIS, JR., and W. S. TILLET, J. Exper. Med. **54**: 587, 1931.

The injection of the type-specific capsular polysaccharides of types I, II and III of *Pneumococcus* into the skin of rabbits actively or passively immunized to one of these types of *Pneumococcus* elicits a type-specific cutaneous reaction. The form of reaction resembles that described by Arthus. The reaction is produced only when type-specific precipitins for the homologous polysaccharide are demonstrable in the blood of the rabbit. In 84 per cent of actively immunized rabbits the serum of which contained type-specific precipitins a reaction was elicited. A positive result was obtained in 100 per cent of rabbits passively immunized with antipneumococcus horse serum, whereas attempts to transfer reactivity from immune rabbit to normal rabbit passively were unsuccessful. In the latter group, the recipients possessed no demonstrable circulating type-specific precipitins. The reaction produced by specific capsular carbohydrates is always associated with well grounded type-specific immunity. A brief summary of the relation of hypersensitiveness and immunity to *Pneumococcus* is given. AUTHORS' SUMMARY.

SERUM SICKNESS IN RABBITS. M. S. FLEISHER and L. JONES, J. Exper. Med. **54**: 597, 1931.

The injection of a single large dose of normal horse serum into rabbits results in the appearance from three to eight days later, of erythematous and edematous reactions on the ears in 68.9 per cent of the animals. The injections may be given by any of several routes, and reactions appear when the site of injection is definitely distant from the ears. Injections of various antisera into rabbits cause the appearance of similar reactions. These reactions can be considered as manifestations of serum sickness in rabbits. AUTHORS' SUMMARY.

ANTIBODIES IN THE SERUM OF RABBITS IMMUNIZED WITH HEAT-KILLED TYPE I PNEUMOCOCCI. E. G. STILLMAN, J. Exper. Med. **54**: 615, 1931.

In rabbits immunized by the injection of suspensions of heat-killed pneumococci, the results obtained as regards not only the development of agglutinating and mouse protective antibodies, but also the persistence of these bodies in the blood, depend to a considerable extent on the route of immunization and the size of the inocula. Agglutinins may appear in the serums of all the rabbits except those inoculated subcutaneously, but in most instances they disappear within a short time. Protective antibodies appear in the serums of all rabbits, no matter which route of injection is employed, and they persist much longer than do the agglutinins. They persist longest when the injections are made intravenously or intraperitoneally and are of briefest duration when the injections are made subcutaneously. AUTHOR'S SUMMARY.

LOCALIZATION OF PNEUMOCOCCI IN THE LUNGS OF PARTIALLY IMMUNIZED MICE FOLLOWING INHALATION OF PNEUMOCOCCI. E. G. STILLMAN and A. BRANCH, J. Exper. Med. **54**: 623, 1931.

When mice are passively immunized by the intraperitoneal injection of antipneumococcus horse serum or actively by the injection of heat killed pneumococcus cultures, and are then alcoholized and sprayed with a culture of pneumococci of the same type as that of the bacteria employed in immunization, a considerable number die with localized lesions in the lungs. If instead of injecting immune serum of the type corresponding to that of the bacteria employed in producing the infection, normal horse serum or immune serum of a heterologous type is

injected, or if the animals are previously immunized by the injection of killed pneumococci of a heterologous type, none of the animals that died show any evidence of localization of the infection in the lungs. The occurrence of pulmonary lesions in alcoholized mice after they have been sprayed with a culture of pneumococci is the consequence of a general immunity of a very mild grade.

## AUTHORS' SUMMARY.

CUTANEOUS REACTIONS AND CIRCULATING ANTIBODIES IN LOBAR PNEUMONIA.  
M. FINLAND and W. D. SUTLIFF, *J. Exper. Med.* 54:637 and 653, 1931.

Patients with type I, II or III pneumococcus pneumonia who had not been treated with antiserum were studied with respect to their cutaneous reactions to specific pneumococcus polysaccharides, circulating agglutinins and protective antibodies for all three types. From one half to two thirds of the recovered patients gave the typical immediate response of wheal and erythema to the homologous type of pneumococcus. All patients tested showed protective antibodies, and almost all showed agglutinins, for the homologous organism. Patients whose pneumonia proved fatal failed to show the cutaneous reaction or circulating antibodies. In those receiving repeated cutaneous inoculations with various types of specific polysaccharide, antibodies differing from the infecting type were present, probably the result of immunization by cutaneous injections. Positive cutaneous reactions to homologous polysaccharides and similar circulating antibodies were found during the first three weeks after the crisis in patients who had not received intracutaneous injections. No heterologous antibodies were found in these patients. Typical cutaneous reactions and circulating antibodies were demonstrated in patients with no recent history of pneumonia. Patients with persistent or latent infections, later fatal, also gave positive reactions. The agglutination test, though less sensitive than the mouse protection test for determining the presence of antibody, is simplest to use in following the course of pneumonia in untreated patients. Cutaneous responses to type-specific, protein-free carbohydrates of types I and II pneumococci have been produced by the intravenous injection of concentrated bivalent antipneumococci serums. A positive cutaneous response to the specific polysaccharide of type II was passively transferred from convalescent patients to a patient with type II pneumonia. Positive cutaneous reactions were usually associated with recovery, and negative reactions with a fatal outcome. Positive reactions in patients treated with concentrated serums occurred twenty-four hours after the first dose. Positive reactions in patients receiving specific antiserum were associated with the presence of mouse protective antibodies and agglutinins in the serum. Of patients receiving repeated inoculations with the specific carbohydrates, serum-treated ones showed more rapid disappearance of immune reactions than patients receiving no antiserum. It is suggested that the antisera administered interfere in some way with the production of antibodies following the intracutaneous injection of the carbohydrates.

EDNA DELVES.

LOCAL SKIN REACTIVITY: ANTIBODY AUXILIARY TO SERUM NEUTRALIZATION OF MENINGOCOCCUS REACTING FACTORS. G. SHWARTZMAN. *J. Exper. Med.* 54:711, 1931.

In this paper there is described an antibody auxiliary to the neutralization of meningococcus factors. The presence of the antibody facilitates studies on the neutralizing potency of antimeningococcus serums. There is also reported a nonspecific neutralizing factor of a heterologous immune serum, which can be differentiated from specific neutralizing antibodies of antimeningococcus serums. Its nature and connection with auxiliary antibody remain to be determined.

AUTHOR'S SUMMARY.

A SEROLOGIC STUDY OF THE POLYSACCHARIDES OF MENINGOCOCCUS, B. ANTHRACIS, B. PROTEUS, B. SUBTILIS and B. MESENTERICUS. J. ZOZAYA, J. Exper. Med. 54:725, 1931.

The meningococcus polysaccharide reacts with a broad precipitable carbohydrate antibody in common with those of B. anthracis, B. subtilis, B. proteus and B. mesentericus. The anthrax and proteus polysaccharides are specific in the higher dilutions of serum. Antianthrax serum contains two different polysaccharide-precipitable antibodies, one specific and the other nonspecific. Agglutinins have no relation to the carbohydrate-precipitable substance, specific or nonspecific. An immunologic method is given for the study of the probable chemical relation or similarity of polysaccharides of different bacteria similar to that given by Heidelberger, Goebel and Avery for a strain of Friedländer's bacillus and Pneumococcus, type II.

AUTHOR'S SUMMARY.

ELECTROPHORESIS EXPERIMENTS WITH THE VIRUS AND PROTECTIVE BODIES OF YELLOW FEVER. M. FROBISHER, JR., J. Exper. Med. 54:733, 1931.

When suspended in slightly alkaline ( $p_H$  7.4 to  $p_H$  7.8) saline dilutions of clear, hemoglobin-free normal monkey serum, the virus of yellow fever from infected monkeys and from infected, but blood-free, mosquitoes usually acts as if it were possessed of a positive electrical charge. The virus tends to assume a negative charge in fluids having a slightly acid reaction. The iso-electric point of the virus seems to be in the neighborhood of  $p_H$  7, possibly ranging from  $p_H$  7.3 to  $p_H$  6.9. Exposure to fluid having a reaction of  $p_H$  5 for three hours appeared to inactivate the virus. In experiments in which the suspending fluid was prepared with normal serum diluted with distilled water and containing a considerable quantity of partly hemolyzed erythrocytes, the virus tended to migrate to the anode. The protective bodies in yellow fever immune serum appear to carry a negative charge in slightly alkaline saline dilutions of serum.

AUTHOR'S SUMMARY.

GRANULOMA-LIKE ALLERGIC INFLAMMATION. F. ROULET, Verhandl. d. deutsch. path. Gesellsch. 26:189, 1931.

Fresh chicken blood was injected intraperitoneally at frequent intervals into guinea-pigs. From fifteen to twenty days after the last injection, the animals received intrapleural injections of from 2 to 3 cc. of antigen. Three different antigens were used; (1) whole chicken blood, (2) washed red corpuscles and (3) plasma. In the sensitized animals, the resorption of the antigen in the pleura was much slower than in the controls. Also the local reaction in the pleura appeared much later after sensitization. Histologically, the local reaction was found to consist of nodular granulomas with round cells, epithelioid cells and giant cells. These structures somewhat resembled tubercles, except that there was a much earlier production of connective tissue in the allergic granulomas.

C. A. HELLWIG.

THE INFLUENCE OF RETICULO-ENDOTHELIAL BLOCKADE ON ANAPHYLAXIS. M. HAENDEL, Virchows Arch. f. path. Anat. 276:22, 1930.

In guinea-pigs that had received intracardiac injections of india ink prior to a sensitizing injection of horse serum, the production of anaphylactic shock required an appreciably larger second dose of the serum than was required in control animals. A similar result was obtained if the sensitizing injection of horse serum preceded the treatment with india ink. The author concludes that the reticulo-endothelial system may play an important part in the formation of anaphylactic antibodies, and that an intact reticulo-endothelial system is necessary for the development of typical anaphylactic shock.

W. SAPHIR.

THE ACTION OF SERUM LIPOIDS IN THE ANAPHYLACTIC EXPERIMENT WITH THE GUINEA-PIG. R. OTTO and H. HOFFMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:233, 1931.

Otto and Hoffmann here furnish a check of the report of Mercier concerning the anti-anaphylactic and desensitizing action of serum lipoids. The protective effect was irregular. However, the value of the check is greatly reduced by considerable deviations from the original technic of Mercier. Serum lipoids alone failed to sensitize.

I. DAVIDSOHN.

CONGENITAL ANAPHYLAXIS IN THE GUINEA-PIG. R. DOERR and S. SEIDENBERG, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:242, 1931.

Reports are confirmed that female guinea-pigs that have received subcutaneous injections of even very small doses of horse serum can give birth repeatedly to anaphylactic descendants during a period up to one and one-half years following sensitization. The young react to extremely small doses of the antigen and lose their reactivity between forty and seventy days after birth, presenting the characteristics of passive anaphylaxis. No explanation is offered for the long interval between sensitization of the mothers and the birth, though it is known that after the ninth week following injection no anaphylactic antibodies can be demonstrated in the mothers' blood. Active sensitization is rarely observed in the young and only when the mothers have been given an injection of a very large dose of the antigen shortly before delivery. In these occasional events, disturbances in the circulatory channels between mother and fetus may be responsible. Normal newborn guinea-pigs may be sensitized, though somewhat irregularly, with very small quantities of horse serum.

I. DAVIDSOHN.

SEROLOGIC STUDIES ON PUTREFACTION OF MEAT. FELIX SULMANN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:265, 1931.

The previously reported nonspecies-specific antigen of putrefaction could not be found in the products of peptic or tryptic digestion. The latter products were used as antigens in a complement-fixation test with an immune serum produced in rabbits treated with decayed meat. In a similar manner, the antigen was found in feces and possibly in urine. The antigen was not soluble in alcohol.

I. DAVIDSOHN.

SEROLOGY OF THE ISOAGGLUTININ SUBGROUPS  $A_1$  AND  $A_2$ . V. FRIEDENREICH, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:283, 1931.

The two subgroups within the group  $A$  found by Friedenreich and Worsaae (*Compt. rend. Soc. de biol.* **102**:884, 1929) are identical with  $A_1$  and  $A_2$  of Landsteiner, whose terminology is adopted. The  $A_2$  is characterized by a lower agglutinability and constitutes about one fifth of the whole group  $A$ . The group  $AB$  is subdivided into  $A_1B$  and  $A_2B$ . The  $A_1$  and  $A_2$  are sharply separated from each other without transitions. The agglutinin anti- $A$  has two fractions:  $\alpha$  and  $\alpha_1$ . Iso-agglutinins consist of numerous fractions, separable according to their avidity and thermal reactivity. The irregular agglutinin  $\alpha_2$  found occasionally in serums  $A_2$  and  $A_2B$  behaves in its thermal reactivity like a "cold agglutinin" and in its specificity like a true iso-agglutinin. It seems to be identical with the lower fractions of the regular  $\alpha_1$  agglutinin.

I. DAVIDSOHN.

HOW IS THE OCCURRENCE OF ISO-AGGLUTININS REGULATED? V. FRIEDENREICH, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:314, 1931.

An analysis of the various hypotheses leads to the conclusion that the "binding hypothesis" of Bernstein is best suited to explain the various phenomena: All

individuals produce both agglutinins  $\alpha$  and  $\beta$ . The one that corresponds with the receptor in the same individual is being fixed by it and eliminated. This conception may be extended to all normal antibodies. The agglutinins are made up of a whole series of partial agglutinins reacting at various temperatures; the elimination of auto-agglutinins by fixation with homologous receptors is therefore limited by the range of body temperature, leaving the "cold-agglutinins" in circulation.

I. DAVIDSOHN.

THE HETEROPHILIC ANTIGENS IN PARATYPHOID BACILLI. KURT MEYER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:331, 1931.

The heterophilic hemolysins produced in rabbits by injections of various members of the paratyphoid group (groups: B, C and Gaertner) showed a strictly specific behavior, being fixed only by strains of the same group or even subgroup. The O (somatic)-antigens of the strains determined the character of the group or subgroup; the H-antigens were of no importance. The heterophilic antigen in the various bacteria (including *B. dysenteriae* Shiga) does not react with the Forssman immune serums produced by injection of organs, while the guinea-pig organs and sheep red cells react with the bacterial immune serums. The explanation is offered that this is due to a combination of the Forssman antigen in bacteria with their specific carbohydrates, limiting their reactivity to immune serums produced by an antigen combined with the same carbohydrate. The hypothesis is to replace an older one of the existence of numerous partial antigens in the heterophilic antigen of the organs of the guinea-pig.

I. DAVIDSOHN.

THE IDENTITY OF THE HOUSE DUST ALLERGEN. A. PEIPERS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:359, 1931.

From a study of fourteen different house dust extracts it appeared that they contained many different allergens. Extracts of house dusts from regions with a large incidence of asthma were particularly efficient for skin tests. When house dust extracts were inhaled by asthmatic persons whose skin gave positive reactions, bronchial symptoms developed.

I. DAVIDSOHN.

EXPERIMENTAL LUTIN PARENCHYMATOUS KERATITIS. S. M. JENALEJEW, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:365, 1931.

In rabbits and guinea-pigs treated with killed cultures of *Spirochaeta pallida* and in rabbits experimentally infected, injections of luetin into the cornea, about two weeks after the immunization was completed, produced a local inflammatory reaction. In guinea-pigs, the reaction was constant; in rabbits, somewhat irregular. After a short interval, a similar change appeared in the cornea of the other eye, which also was constant in guinea-pigs, but which occurred only in some of the rabbits.

I. DAVIDSOHN.

THE RETICULO-ENDOTHELIAL SYSTEM IN IMMUNITY IN RELAPSING FEVER. I. L. KRITSCHESKI and P. L. RUBINSTEIN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:372, 1931.

Splenectomy alone and in combination with blockade of the reticulo-endothelial system did not decrease the immunity in mice that had become resistant as a result of a previous infection. Animals not previously infected or those that developed no immunity despite infection succumbed to the disease when the spirochetes were injected after splenectomy or blockade, or a combination of the two.

I. DAVIDSOHN.

## Tumors

ULTRAFILTRATION OF THE AGENT OF CHICKEN TUMOR No. 1. WILLIAM MENDELSON, C. E. CLIFTON and M. R. LEWIS, *Am. J. Hyg.* **14**:421, 1931.

The active principle of chicken tumor no. 1 readily passed through 0.5, 1, 2 and 3 per cent collodion membranes. In one instance it passed the 5 per cent collodion membrane. The filtrates that passed through the 4 and 5 per cent collodion membranes were free from demonstrable protein. The size of the particles of the agent of chicken tumor no. 1 was found to be less than 50 millimicrons and possibly less than 15 millimicrons.

AUTHORS' SUMMARY.

IMMUNITY TO WALKER'S RAT SARCOMA No. 1. F. DONAGHY and R. R. HYDE, *Am. J. Hyg.* **14**:495, 1931.

Study of three races of rats revealed that the one (Philadelphia) is susceptible to Walker's rat sarcoma no. 1, whereas two other races of rats are immune in a high percentage of cases. Attempts to immunize the susceptible Philadelphia rats were made (1) by removing the implant after it had grown in the host for some time; (2) by feeding tumor tissue; (3) by feeding tissues from resistant rats; (4) by the injection of immune serum from the rabbit and the guinea-pig; (5) by injection of serum and whole blood from chickens treated with sarcoma no. 1; (6) by treatment with embryo skin; (7) by treatment with an emulsion of spleen from tumor-bearing animals; (8) by passage of tumor fragments through the resistant host, and (9) by nursing the young of the susceptible Philadelphia stock on tumor-bearing mothers. In all cases the animals remained susceptible to Walker's rat sarcoma no. 1.

PAUL MERRELL.

IMPLANTATION PERITONEAL CARCINOMATOSIS OF OVARIAN ORIGIN. J. A. SAMPSON, *Am. J. Path.* **7**:423, 1931.

Implantation peritoneal carcinomatosis arises from the repair of injuries to the peritoneum caused by cancer cells which have escaped into the peritoneal cavity and lodged on the surface of its serous membrane, together with the continued growth of these cells in this situation. The various stages in this repair, as well as the laws governing the same, are similar to those encountered in the repair of tissues injured by foreign bodies, and in the taking of skin grafts, namely, the healing of wounds. The histologic structure of these implants varies with the reaction of the peritoneal tissues before and after the fixation of the cancer cells and the activity of the latter. As a result, cancer becomes embedded in the peritoneal scar, encapsulated on its surface and enmeshed in adhesions, or, like the epithelial growth of a successful skin graft, spreads over the peritoneum without encapsulation. The malignant cells of these metastatic tumors possess the same potentialities of invasion and dissemination as those of a primary cancer.

AUTHOR'S SUMMARY.

THE NATURE OF HODGKIN'S DISEASE. E. M. MEDLAR, *Am. J. Path.* **7**:499, 1931.

Evidence is presented which suggests that Hodgkin's disease is a malignancy of the bone marrow. The type cell appears to be the megakaryocyte. The developmental cycle of the megakaryocyte is presented. It would seem that the typical megakaryocyte is the result of fusion of several premegakaryocytes. The histopathology of Hodgkin's disease is a pleomorphic aggregation of cells which represent the developmental cycle of the megakaryocyte. It is not essential to have fibrosis or eosinophilic or neutrophilic infiltration to establish the diagnosis of Hodgkin's disease. The involvement of lymph nodes and other tissue outside

of the bone marrow appears to be metastatic tumor growth. Evidence is presented which tends to prove that all blood cells arising from the marrow have a common parent cell. The term "megakaryoblastoma" is suggested to designate true Hodgkin's disease.

AUTHOR'S SUMMARY.

THE EFFECT OF TESTICLE EXTRACT ON THE ROUS SARCOMA. D. C. HOFFMAN, FREDERIC PARKER, JR., and T. T. WALKER, *Am. J. Path.* 7:523, 1931.

Rabbit testicle extract markedly enhances growth of the Rous sarcoma (chicken tumor I) in chickens. This effect is the same whether tumor mash or a cell-free filtrate of the tumor is used in the inoculations. Rooster testicle extract causes no enhancement. Normal rabbit serum causes a slight degree of enhancement.

AUTHORS' SUMMARY.

TUMORS OF THE EXTRAHEPATIC BILE DUCTS. P. F. SHAPIRO and R. A. LIFVENDAHL, *Ann. Surg.* 94:61, 1931.

Fifteen tumors of the extrahepatic bile ducts were encountered in 2,500 necropsies. Of these one was an amputation neuroma, one a solid adenoma, one a congenital cyst of the cystic duct and twelve carcinomas. According to this material, carcinoma of the extrahepatic bile ducts was twice as common as carcinoma of the gallbladder and three times as common as cancer of the head of the pancreas and comprises 3.7 per cent of all cancers. In the cancers the clinical course for those in the extrahepatic bile ducts averaged 5.3 months; 60 per cent occurred in males, and 71 per cent in white persons, at an average age of 59.7 years. The percentage figures for males, etc., are corrected sex and race differences. Twice as many necropsies were held on males as on females, and twice as many on white people as on colored. Contrary to the usual opinion, metastases occurred frequently and early and often were extensive so as to dominate the clinical picture. No difference was noticed between scirrhous or polypoid tumors in their tendency to intramural growth, their histologic structure or their tendency to metastasize.

GEORGE RUKSTINAT.

THE GANGLIONEUROMAS OF THE CENTRAL NERVOUS SYSTEM. BERNARD J. ALPERS and FRANCIS C. GRANT, *Arch. Neurol. & Psychiat.* 26:501, 1931.

Alpers and Grant describe a case of ganglioneuroma, a tumor classified by Virchow as true neuroma but lately as ganglioglioma and gangliogliioneuroma. The patient was a boy, aged 16, who had suffered from paroxysmal frontal headaches and later from attacks of blindness of one or two minutes' duration. The boy finally became blind. He had frozen pupils and signs of a brain tumor, which was localized in the pituitary region with endocrine disturbances, soft, smooth skin, hips of the feminine type, a feminine distribution of the pubic hair, only little axillary hair and rather prominent breasts. The tumor was partially removed. It contained numerous ganglion cells, some of which were apolar, some bipolar or multipolar. There were many small cells, evidently glia, numerous blood vessels and nerve fibers, mostly nonmedullated. The cells in ganglioneuromas are regarded not as neuroblasts but as immature ganglion cells which for some reasons failed to develop.

GEORGE B. HASSIN.

BASOPHILIC ADENOMA OF THE HYPOPHYSIS WITH ASSOCIATED PLURIGLANDULAR SYNDROME. HAROLD M. TEEL, *Arch. Neurol. & Psychiat.* 26:593, 1931.

The anterior lobe of the hypophysis is known not only to possess gonad-stimulating properties but also to have some influence on other endocrine organs, such as the suprarenal cortex and thyroid. The substance responsible for the

gonad-stimulating effect is elaborated in basophilic cells of the anterior lobe. As the case reported by Teel shows, a basophilic adenoma of the hypophysis may be associated with hypertrophic changes in the endocrine organs. A white woman, aged 20, had convulsions and became unconscious, after two days of chills, fever and vomiting. The weight was increased (206 pounds), the basal metabolism was plus 42, and there was excessive growth of hair on the face, thorax, abdomen and extremities. The patient died of cerebrospinal meningitis. The hypophysis showed at its lateral inferior margin a nodular collection of basophilic cells measuring 2.5 mm. in diameter. The surrounding glandular tissue was compressed and atrophied. The thyroid, thymus and suprarenals were enlarged, the pancreas showed an unusually large number of islets, and the ovaries, which were much larger than usual, contained numerous small follicular cysts.

GEORGE B. HASSIN.

GLIOMAS OF THE RETINA. ROY R. GRINKER, Arch. Ophth. 5:920, 1931.

Heretofore, cellular studies of retinal gliomas have failed to reveal the exact type of glia cells within tumors because sections embedded in celloidin and stained with hematoxylin-eosin have been almost exclusively used. Fixation in formaldehyde and silver impregnations of frozen sections are essential to demonstrate the cell processes and their relations to blood vessels and each other. With these methods gliomas of the retina possibly can be classified according to the normal histogenetic stage of the retina. Such a classification may be utilized in correlation with the clinical and biologic characteristics of the component cells to great advantage.

The glial tumors of the retina now known are divisible into three large groups. The medullo-epitheliomas consist mostly of primitive retinal epithelium, which persists in adult life as ciliary epithelium and from which these tumors arise, and also of neuro-epithelium and retinoblasts. Retinoblastomas are chiefly composed of retinoblasts. In the tumors composed of these indifferent cells, evolutionary stages in the histogenesis of adult retinal glia have been found, but no cells of the ganglion series have as yet been described. In the neuro-epitheliomas, spongioblasts are found in rosette-like arrangement. These cells closely resemble the rods and cones. Primitive spongioblasts, astroblasts, astrocytes and oligodendroglia are also found, all normally derived from the neuro-epithelium. A histogenesis of glia possibly occurs in these tumors, but each group contains a preponderance of the more primitive types of cells. Tumors of pigmented epithelium have not been described, possibly because the epithelial cells lose their pigment in neoplasms. Other types of tumors probably will be revealed with further study of more material.

AUTHOR'S ABSTRACT.

THE EFFECT OF TISSUE EXTRACTS ON THE RESPIRATION OF TUMOR TISSUES. L. J. SOFFER, Bull. Johns Hopkins Hosp. 49:320, 1931.

The accelerating effect of rat liver extract and to a lesser extent of kidney extract on the oxygen consumption of mature erythrocytes has been confirmed. Methylene blue (methylthionine chloride, U. S. P.) increases the oxygen consumption of tumors (of carcinomas more than of sarcomas), while it exercises no such effect on normal tissues. Liver extract, homologous and heterologous, fails to increase the respiration of either tumors or normal tissues.

AUTHOR'S SUMMARY.

THE MELANOMAS OF GREY AND WHITE HORSES. S. HADWEN, Canad. M. A. J. 25:519, 1931.

The pigmentary systems of horses and man differ. Animals are entirely clothed with hair and shed it annually. Horses that whiten with age are predisposed to melanomas. The tumors progressively increase in size with age. This is associated with continued melanin production, though the hair no longer makes



use of it. The melanomas begin to form when horses are still young, 6 years or over. The deposits are found in avascular places. They are common in the perineum. Other abnormalities in color occur on the line of union between the two halves of the body. White marks are strongly inherited. The commonest sites for melanomas are in the regions of the tail and mane. It is improbable that this is due to irritation from the harness, as has been suggested, but more likely that a larger flow of tyrosine is being directed to these parts. Freckles in horses depend on exposure to sunlight, length and color of hair and to age. As they ascend to the corneum, the columnar basal cells of the epidermis lose their strands of melanin and alter their shape. At the edge of leukodermic areas the basal cells produce melanin irregularly; some overproduce; others are unable to form pigment. Melanic masses may form extensions through pressure from outside the body. The sweat glands may be destroyed by melanin, but the subaceous glands are not infiltrated. In deep situations the melanoblasts are spindle-shaped, having long processes and coarser melanin than one finds in the epidermis. Many types of cells, such as the basal cells, are round when no pressure is exerted on them. It is believed that many varieties of so-called atypical cells are in reality normal cells that have been molded out of shape through pressure or the release of pressure exerted by edematous fluids or overgrowth. Through overproduction of melanin, the basal cells of the epidermis may become disarranged and appear atypical. Melanomas in horses rarely become malignant.

AUTHOR'S SUMMARY.

THE BLOOD OF NORMAL RABBITS AS AN INDEX OF THEIR RESISTANCE TO A TRANSPLANTABLE NEOPLASM. A. E. CASEY and L. PEARCE, *J. Exper. Med.* 54:475, 1931.

The blood cytology of ninety-one rabbits was studied prior to inoculation with a transplantable malignant neoplasm. The following statements refer in each instance to the mean values of the preinoculation counts. The animals that were most resistant to the malignant disease had, before inoculation, normal red and white cell counts, normal hemoglobin percentages, high eosinophil counts and low counts of monocytes and lymphocytes. The relations of the neutrophil and basophil counts were irregular, but normal values also appeared to be associated with greater resistance. The most susceptible animals were those that had, before inoculation, red cell counts above 5,500,000 or below 5,000,000 per c.mm.; hemoglobin above 70 per cent or below 60 per cent (Newcomer); white cell counts below 6,000 or above 8,500 per c.mm.; low eosinophil, high monocyte or high lymphocyte counts. No animal with any of the following findings prior to inoculation recovered completely from the tumor as determined by autopsy examination, red cells above 5,500,000 per c.mm. of blood; hemoglobin above 70 per cent; total white cells above 10,000 per c.mm.; eosinophils below 120 per c.mm., or below the relative value of 1.5 per cent; basophils below 400 per c.mm., or below the relative value of 6 per cent; lymphocytes above 3,600 per c.mm.; monocytes above 1,500 per c.mm.; neutrophils above 5,000 per c.mm., and total granular cells above 5,700 per c.mm. In the case of each the following preinoculation values, only one animal was completely free from tumor at autopsy: hemoglobin below 60 per cent, red cells below 4,800,000 per c.mm., total granular cells below 3,300 per c.mm., total nongranular cells below 2,300 per c.mm. and total nongranular cells above 3,700 per c.mm. No animal with preinoculation eosinophils above 3.9 per cent or basophils above 16 per cent died from the tumor. The blood findings before inoculation could be related to the character and outcome of the malignant disease, from the standpoint of animal groups as well as in the case of individual rabbits. From the results of the experiments here reported, it seems possible to predict with an accuracy of between 80 and 90 per cent the individual resistance or susceptibility of rabbits to the tumor by a study of their blood cells before inoculation.

AUTHORS' SUMMARY.

EPITHELIOMA AND SARCOMA OF THE STOMACH. A. KLEINKNECHT, C. OBERLING and S. TASSOWATZ, *Bull. Assoc. franç. p. l'étude du cancer* **20**:209, 1931.

If one is to judge from the cases reported in the literature, multiple tumors of this kind have a predilection for certain organs such as the uterus, the breast, the esophagus and the thyroid gland.

In the case reported, it concerned a linitis plastica and a leiomyosarcoma. The topographic relationship of the two tumors was very intimate, and the authors believe that the gastric cancer has led to the sarcomatous transformations of the mesenchymal element of the stomach with which it came in contact.

B. M. FRIED.

ANGIOMA OF PIA OF CEREBELLUM. A. DE BLASI, *Pathologica* **23**:18, 1931.

A case of a tumor in the pia mater of the cerebellum in a man, aged 74, is reported. The histologic picture was that of capillary angioma with numerous nests of small round cells between the capillaries. The origin of the cell nests could not be determined.

E. HAAM.

PRODUCTION OF TUMORS IN URINARY BLADDER IN RATS. E. PUCCINELLI, *Pathologica* **23**:73, 1931.

In twenty-six rats, a bolus consisting of tar, paraffin and scarlet red was introduced into the urinary bladder. The animals were subsequently given subcutaneous injections of solutions of arsenous acid and tar. In three cases, papillomatous growths showing keratinization were observed, but in no case did a malignant tumor develop.

E. HAAM.

PAPILLARY CYSTADENOMA OF THE PANCREAS. F. TAVERNARI, *Pathologica* **23**:207, 1931.

The author reports a case of papillary cystadenoma, without signs of malignancy of the pancreas, in a woman, 72 years old. The genesis of the tumor was probably due to a congenital abnormality of the cells of the excretory ducts.

E. HAAM.

ARE BIOPSIES ON MALIGNANT TUMORS DANGEROUS? A. EPSTEIN and A. FEDOREJEFF, *Arch. f. klin. Chir.* **165**:357, 1931.

Between October, 1926, and June, 1930, 1,581 biopsies were made at the Oncologic Institut in Leningrad. In 1,222 cases this was done once, and in 359 patients repeatedly. The clinical diagnosis of malignancy was substantiated by the pathologist in 81.7 per cent.

Fever after biopsy was observed in 4 per cent of 1,226 cases of carcinoma and in 19 per cent of 53 cases of sarcoma. In two cases biopsy was followed by severe infection. Fatal infection occurred in one case of carcinoma of the uterus with extensive metastases. In three carcinomas and in one sarcoma the diagnostic incision seemed to accelerate growth; however, all these four cases were late forms with ulceration and in the two carcinomas of the breast there was also widespread metastases before biopsy. Very slight hemorrhages, easily controlled by gauze, occurred nineteen times; no severe hemorrhage was observed in the whole series of biopsies. To avoid any possible danger, the authors recommend the electric knife for diagnostic incisions, and they cauterize the wound with concentrated phenol. The immediate diagnosis from frozen sections during operation is regarded as the method of choice. In accessible tumors, the enlarged regional lymph glands are excised for diagnosis. In oral and laryngeal cancer, radiation treatment is initiated before biopsy.

The authors conclude from their observations and from a review of the literature that complications after well performed biopsy are exceptional. There is some danger of acceleration in growth following biopsy, especially in sarcoma. If ever possible, radical operation should follow immediately the diagnostic incision. In tumors of the breast, biopsy should not be performed as an independent operation, but the histologic diagnosis should be made during operation from frozen sections. Radiation treatment, if indicated, should precede the diagnostic incision. In tumors covered with intact skin, enlarged regionary lymph glands may be excised without danger for diagnostic purposes.

C. A. HELLWIG.

### Medicolegal Pathology

THE RELATION OF PATHOLOGY TO LEGAL MEDICINE. Z. E. BOLIN, California & West. Med. **35**:195, 1931.

Bolin contrasts the unsatisfactory state of legal medicine in this country with the organization and importance of medicolegal science in Europe as a university discipline and as a function of the state. He suggests a plan of correlated organization of the essential elements of existing university activities into a provisional university department of legal medicine. Such a department should, at least at first, be a subdivision of the department of pathology, because of the dominant share of this science in medicolegal work. It should be under the direction of a pathologist, whose duty it would be to correlate the necessary activities of other medical and university departments into an organization capable of providing adequate instruction in the subject. One or more graduates in law could present the legal aspects. The facilities of the department would be offered to the police and coroner, whose material should be made available to the department for teaching purposes. The teaching could be condensed within a single year or spread over the four years of the medical course. The organization proposed would entail no great expense on the university, because it would make use of personnel and facilities that the university has. By the time that the duties of the department of legal medicine had become more onerous, it would have established its claim to more liberal financial support by service rendered in teaching and in the administration of justice.

O. T. SCHULTZ.

UNEXPECTED AUTOPSY RESULTS IN UNEXPECTED DEATHS. W. J. DEADMAN, Canad. M. A. J. **25**:317, 1931.

From a series of about 1,200 autopsies over a period of ten years, the following cases are presented in view of the unexpectedness of the deaths and the unusual autopsy results, together with as much of the clinical history and findings as possible: extreme fatty degeneration of the myocardium in a 17 year old girl, following arsenical therapy in syphilis; traumatic hemato-pericardium following a traumatic, nonbleeding puncture wound of the chest; aortic stenosis in a 45 year old man with no history of previous illness; congenital absence of the ventricular septum in a 3 day old infant; coronary thrombosis in a 14 year old girl following violent exercise, the origin of the thrombosis being vegetative masses at the mouth of the left coronary artery; lobar pneumonia in a workman who collapsed on duty; influenzal pneumonia, hemorrhagic, in a middle-aged man who collapsed at work; glioma of the cerebellum in a patient who died during a diagnostic spinal puncture; hemorrhage into a cerebral cyst following a slight contusion of the head in a young man of 24; death by suffocation in epilepsy following an attack in a man in whom epilepsy had never been suspected; appendical hemorrhage in which a "common pin," evidently swallowed, had eroded through a small vessel and the appendical wall, causing hemorrhagic peritonitis; infected Meckel diverticulum as the point of incitement of "idiopathic" peritonitis; traumatic rupture of the spleen following a fall from a horse, in a young soldier who had had previous attacks of malaria; "status thymicolymphaticus" in a 12 year old boy

who had apparently been drowned following a blow from a row-boat oar which caused him to fall overboard; rupture of the posterior wall of the duodenum in a person who was crushed against the wall of a sand-pit by the hub of a wagon wheel.

WILLIAM FREEMAN.

SUDDEN DEATH IN ASYMPTOMATIC SUBACUTE BACTERIAL ENDOCARDITIS. G. L. WEST, *New England J. Med.* **205**:675, 1931.

A woman, 31 years of age, an expert swimmer and athletic instructor, while playing tennis, became suddenly weak, cyanotic, then unconscious and died within an hour. Post mortem, many friable endocardial vegetations were found. At the bifurcation of the left coronary artery, there was an embolus from a vegetation, triangular in shape and 10 by 5 by 5 mm., occluding both branches of the artery distal to the bifurcation. Cultures from the vegetative growths and the embolus gave gram-positive cocci, occurring singly and in chains.

WILLIAM FREEMAN.

A CASE OF POISONING BY SODIUM NITROPRUSSIDE. F. S. FOXWEATHER, *Brit. M. J.* **2**:344 (Aug. 22) 1931.

This is the first recorded death by poisoning with sodium nitroprusside. The patient soon became unconscious; respirations were slow and irregular, with heavy and short inspirations and prolonged expirations. The pupils reacted sluggishly and were moderately dilated. There were subnormal temperature and slow pulse. Death occurred in two and one-half hours after ingestion of the poison. There were no convulsions. Postmortem examination elicited a marked odor of hydrogen cyanide gas on opening the body. The gastric contents gave off a hydrogen cyanide odor. The condition of the tissues was consistent with hydrogen cyanide poisoning.

WILLIAM FREEMAN.

AUTOPSIES OF UNUSUAL INTEREST. I. B. MORRIS, *Lancet* **2**:737, 1931.

The author presents several instances of unusual conditions found at autopsy in cases of sudden death, namely, spontaneous rupture of the aorta in a man aged 49; primary carcinoma of the bronchus with extensive invasion of the lung and mediastinum with rupture into the pericardium in a man aged 42; impacted gallstone in the ampulla in a 65 year old woman; acute extensive miliary tuberculosis in a man aged 61.

WILLIAM FREEMAN.

SUDDEN DEATH FROM INTERSTITIAL CARDIAC HEMORRHAGE. F. MOOR, *Lancet* **2**:740, 1931.

In a woman, aged 68, who eight months prior to death had pain in the chest, apparently due to bruising of the right wall of the chest, the only other sign found was a moderately hypertrophied heart and hypertension. During the succeeding interval, the patient enjoyed fair health and died suddenly. The heart revealed calcareous deposits and stenosis of the proximal ends of each coronary vessel so that a probe tip could be passed, but no thrombosis. The lower right portion of the interauricular septum showed rather extensive areas of hemorrhage beneath the endocardium and elevating it for a short distance. The hemorrhage appeared to be of too recent origin to be related to the previous accident.

WILLIAM FREEMAN.

INHERITANCE OF PAPILLARY PATTERNS OF THE PALM AND FINGERS. B. MUELLER, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:407, 1931.

In cases of questionable paternity, in addition to the blood grouping tests, the analysis of the papillary lines of the fingers and of the palmar ridges may, under

certain favorable conditions, be used to advantage. The Gruenberg theory in regard to the inheritance of papillary arch formations is critically discussed and evaluated. The important work of Poll and his classification of various patterns are emphasized, since he seems to prove that, in particular instances, by a comparison test of papillary patterns of persons involved (mother, child and questionable father), the accused man may be excluded as parent of the child.

E. L. MILOSLAVICH.

URIC ACID INFARCTS IN THE STILLBORN INFANT. M. BOGEN, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **17**:426, 1931.

Ever since the first observations of Virchow (1847), it has been thought that uric acid infarcts are found only in new-born infants who have breathed, and consequently a certain medicolegal significance has been attached to such infarcts. But as far back as 1900, the Hektoen and Riesman textbook emphasized that such infarcts occur also in stillborn infants. The appearance of uric acid infarcts in kidneys of infants who die shortly after delivery seems to have a certain relation to uric acid formation due to disintegration of large numbers of leukocytes, since in the first days of extra-uterine life there is pronounced leukocytosis. The author describes a pertinent case of a full term but stillborn child, whose dead body was delivered by means of cephalotripsy. Because of the protracted delivery in this case, it is assumed that, as demonstrated in such instances by Schwarz and Mausloff, the umbilical blood contained a large amount of uric acid, which, in connection with disintegration of large numbers of leukocytes, might cause the infarcts, regardless of whether the child was born alive or dead.

E. L. MILOSLAVICH.

### Technical

PRESERVATION OF HEARTS BY PARAFFIN INFILTRATION. L. GROSS and E. LESLIE, *Am. Heart J.* **6**:665, 1931.

The interior of the unopened heart is first examined with a nasoscope, and postmortem blood clots are removed. Fixation is accomplished by perfusion of the organ with neutral formaldehyde solution for seventy-two hours through a system of cannulas by which, also, the heart is suspended in the formaldehyde solution and filled with it until the walls take their natural shape. After dehydration for from two to four days in increasing percentages of alcohol, the heart is put through two changes of toluene in seventy-two hours; it is then placed in a bath of 5 parts of beeswax and 95 parts of paraffin at 56 C., renewed once in forty-eight hours. While the heart is still warm, incisions are made, allowing exposure of the interiors of the chambers by removal of their outer walls. The coronary arteries are opened during the dehydration in alcohol. Blocks for microscopic study are removed after fixation in formaldehyde or, preferably, after paraffin infiltration, while the tissues are still warm.

E. M. BARTON.

PREVENTING DEHYDRATION OF CULTURE MEDIUMS. W. KRANTZ, *Dermat. Wchnschr.* **93**:1263, 1931.

The medium is placed on the bottom of a small Erlenmeyer flask fitted with a cord tightly sealed in with paraffin. A narrow glass tube of inverted U shape is fitted into the single hole in the cork and also sealed in with paraffin. Cultures have been kept for five years without dehydration of the medium. A tiny drop of water of condensation collects on the tip of the tube in the flask.

LAWRENCE PARSONS.

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Nov. 14, 1931*

LEILA CHARLTON KNOX, President, in the Chair

### A CASE OF GENERALIZED NECROSING ARTERITIS. CHARLES T. OLCOTT.

A 12 year old white girl of English extraction had symptoms pointing to pyelitis eleven months before admission and pharyngitis one month before admission. Her heart seemed enlarged. The temperature rose almost daily to 103 F. The red blood cells were 3,000,000, with 40 per cent hemoglobin; the white cells were 20,000, with 84 per cent polymorphonuclear leukocytes. The urine showed albumin in large amounts and many pus cells. The blood urea nitrogen rose from 50 to 106 mg. per hundred cubic centimeters. Blood cultures were negative, but one urine culture showed nonhemolytic streptococci. The patient vomited persistently; the urinary output decreased; convulsive attacks developed, and she died three weeks after admission. The Wassermann reaction was negative. The clinical diagnosis was nephritis.

At autopsy there were edema of the lungs and ascites. Reddish zones, about 2.5 cm. in diameter, were found on the surface of the liver, and in these there were elevated white masses of pinhead size. The kidneys were mottled, partly dark red and partly light red. The darker red areas contained elevated white masses similar to those in the liver. These were found on the surfaces and in the deeper portions of the kidneys. No gross findings demonstrated the nature of the process.

Histologic study (illustrated by lantern slides), however, demonstrated marked changes in the arteries of the liver, kidney, appendix, heart and pleura and in a branch of the abdominal aorta. These showed hyalinization and thickening of the medial coats with, in some cases, destruction of both elastic laminae. Marked infiltration of the adventitial layers by polymorphonuclear cells, lymphocytes and eosinophils was present. Intimal changes varied in degree. In some cases, notably in the kidney, intimal proliferation markedly diminished the lumina of the vessels, while in others the intima was normal. No thrombosis or aneurysmal dilatation was found. Associated with the vascular lesions of the kidney and liver were hemorrhagic infarcts. The cytoplasm of some of the liver cells and some of the muscle fibrils of the heart showed bluish staining in hematoxylin and eosin preparations.

A postmortem blood culture was positive for an hemolytic streptococcus, but this was considered as probably a late invader. Bacterial stains of the tissues failed to demonstrate any specific etiology. This is in accordance with the observations of the majority of observers. A hypersensitization phenomenon cannot be excluded.

*Summary.*—A case of necrosing arteritis of renal, hepatic and other vessels is presented. Presumably because of the patient's rapid death, there was no development of the nodular aneurysmal dilatations characteristic of periarteritis nodosa. In other respects the case belongs in that group.

### DISCUSSION

MORGAN VANCE: I was much interested in Dr. Olcott's case as an example of periarteritis nodosa without the nodosities. The majority of cases are a mixture of necrotic arteritis and aneurysm formation in some of the small vessels in different parts of the body, namely, in the heart, liver, kidneys, mesentery, intestines

and elsewhere. The disease is a rather complicated one, and I do not wish to take time to discuss it in detail, but I have three lantern slides of a case Dr. Graham and I reported recently in *THE ARCHIVES* (12:521, 1931). This case is one of the more orthodox types of periarteritis nodosa, in that it shows multiple aneurysms and also the formation of multiple infarcts in the liver and kidneys.

PAUL KLEMPERER: We have made some observations on this subject in the last years at the Mount Sinai Hospital, and Dr. S. Otani has collected most of our cases for the exhibit at the recent Graduate Fortnight of the New York Academy of Medicine. We have had eleven cases in the last five years; nine of them and an earlier case that Dr. Otani included were shown. The most interesting fact is that the number of cases has increased so remarkably in the last ten years. Up to then individual cases were reported because the condition was considered unusual enough to justify the report of a single case. In the last few years, the number of reports has gone up markedly. I think this is due to the fact that we reserve the diagnosis periarteritis nodosa not only for cases in which the arterial lesion is classic, according to the original description by Kussmaul and Mayer, but also—and correctly—for cases of generalized necrosing arteritis without aneurysm formation. I think one should abstain from referring to this type of vascular disease as periarteritis nodosa, because the actual gross lesion is not so frequently found and is not always so conspicuous as it is said to be in the original description. In the group of cases that Dr. Otani showed there were only three cases in which aneurysms were seen so that the diagnosis of periarteritis nodosa in the classic sense was justified. In one of these, aneurysms about the size of a cherry and even larger were shown in the kidney. In other cases, arterial lesions could be seen with the naked eye only with difficulty. However, one could easily recognize them if one used a magnifying glass on the gross specimen. The use of a magnifying lens was indicated because of multiple infarcts in the kidneys and other organs that were apparently not of embolic origin. In all these cases, the appearance of the infarct-like lesions suggested a search for a primary vascular change. In nearly every one of these instances, arterial lesions could then be demonstrated in the gross. Macroscopically, they did not impress one as aneurysms so much as focal thickenings of the arteries. The interlobar arteries of the kidneys showed lesions frequently. We nearly always found them in the mesenteric arteries just at the insertion of the branch of the mesenteric artery into the intestine. One place in which we looked for them was the gallbladder, in which they were present frequently. I think that by routine histologic examination one would find much more necrosing arteritis than would be suggested by the gross appearance. Aneurysms are not so frequent, but the necrosing arteritis demonstrated on minute examination of the gross material, or only histologically, is not so rare.

Most interesting, it seems to me, is the relationship that exists between renal symptoms and necrosing arteritis. In Dr. Olcott's case, the renal symptoms were the leading ones; there was apparently no nephritis. However, it is peculiar how frequently diffuse glomerulonephritis is combined with generalized necrosing arteritis. In three of our cases there were diffuse glomerulonephritis of typical appearance and generalization of the arterial disease. This is particularly interesting because glomerulonephritis shows not infrequently (in about from 10 to 20 per cent of cases) in the subacute stage a lesion in the smaller arteries, the afferent vessels, that is identical with the necrosing lesion of the generalized type described. This might throw some light on the pathogenesis of the disease; that is, that it is of the same pathogenesis as glomerulonephritis, namely, toxic. I realize that one usually has not paid particular attention in this disease to the history of the patient in regard to the question of antecedent infection, which if found would suggest that a peculiar stage of sensitization has been caused by the previous infection, which at the recurrence of infection, perhaps identical or of different etiology, leads to this peculiar necrosis of the blood vessels. It might be worth while to mention in this respect two cases of rheumatic fever with Aschoff bodies in the myocardium and verrucous endocarditis in which systemic

necrosing arteritis was found. In both cases there were certain clinical peculiarities that caused the clinicians to doubt the diagnosis of rheumatism, though there was otherwise considerable evidence in favor of it. In both cases, during the progress of the disease, pain was particularly conspicuous in various parts of the body, which is not, as far as I am informed, considered typical of rheumatic heart disease. In both instances there existed a generalized arterial disease of the same type as that described tonight. In one case, it was found only on close study of the histologic sections; in the other case, in which the disease of the arteries was particularly conspicuous in the lungs, the lungs were riddled with small nodules, arranged around the blood vessels. I think such findings of necrosing arteritis in rheumatism are particularly interesting because of the reports of von Glahn and Pappenheimer on arteritic lesions in rheumatism. I have always thought that they were not specific for rheumatism but had something to do with the peculiar evolution of rheumatism, especially with the recurrent type of disease that rheumatism is.

I feel that we should not look for a specific bacteriologic etiology in necrosing arteritis, but should search for a specific immunologic phase or a specific sensitiveness of the blood vessels, and try to reproduce this, if possible, experimentally, instead of trying to reproduce the disease by the injection of organ extracts in such cases.

Two other cases suggest a similar pathogenic principle. In both instances, there had existed a severe sinusitis for a long time, and the terminal disease, proved at autopsy to be general necrosing arteritis, had begun shortly after some operation on the nasal sinuses. It was possibly due to this operation that a new invasion of toxins into the blood stream had been caused.

In regard to the clinical diagnosis in our eleven cases, the diagnosis was made during life only once. This was in a case in which there were numerous large aneurysms of the kidneys, causing severe hematuria. In this instance, Dr. Edwin Beer suggested the diagnosis of periarteritis nodosa. In the other cases, the diagnosis was made only at the autopsy table.

ALFRED PLAUT: Dr. Klemperer's remarks strongly emphasize the nonspecific nature of periarteritis nodosa. Several years ago, I was struck by peculiar lesions of the arterioles in the vermiform appendix that resembled periarteritis nodosa. These lesions were demonstrated at a meeting of the American Association of Pathologists at Albany. Meanwhile the same lesions have been found in many routine specimens of the appendix vermiformis and occasionally in specimens of the internal female genital organs. They may be designated as focal, nonspecific arteriolitis. I have never seen them in veins or in any medium-sized or large arteries thus far. This focal disease occurs in young people as well as in older people. It is found in inflamed appendixes and in the so-called normal appendixes taken out in the course of abdominal operations. It is located in the muscle coat and in the serosa of the appendix. In the fallopian tube, it has been found in the outer layers only. I have not seen it in any other organ of the body except, perhaps, in one subacutely inflamed inguinal lymph node.

The similarity of the lesion to that of small arteries in periarteritis nodosa is striking. Some of my own photomicrographs could almost be substituted for the pictures that Dr. Olcott showed tonight.

The distribution of the lesion is very irregular. It generally occupies only small parts of an arteriole. By making serial sections and reconstructing, one finds that, for instance, one small branch of an arteriole may be involved for a distance of a fraction of a millimeter, being normal above and below that point, and with all other branches in the same region being normal.

Thus it is easily understood that no disturbance of the tissue due to the closure of these small vessels can be found. Theoretically, however, I consider this lesion of great importance. Since it is found in tissues removed from all kinds of patients without relation to any specific infection and without relation to age, the best way out of our ignorance may be to surmise that some immunologic condition of the tissues may be responsible for the pathologic change in the arterioles.



The lesion always seems to begin with a hyaline deposit under the endothelium. The difficulty of having a distinctly focal disease caused by an immunologic change certainly exists. Our hypothesis shares that fault with many others.

It seemed to me incredible that a characteristic morphologic change in the vermiform appendix requiring no special methods for finding it should have escaped detection so far. I therefore have hesitated to come forward with it.

FACTORS INFLUENCING ERYTHROCYTIC SEDIMENTATION. THOMAS H. CHERRY and (by invitation) JOHN A. KILLIAN.

The tables presented show that there are two factors that apparently influence the rate of sedimentation. The first is the physical and visible elements; the cross-sedimentation experiments indicate that elements in the plasma definitely influence the rate. The cell volume and total solids indicate that the smaller the volume and the diminution in solids the faster is the rate of sedimentation. The second factor is the chemical one. An increase in the sugar concentration diminishes the rate of slow sedimentation, and has no effect on the rate of fast sedimentation. The total protein remains unchanged. Variations in the protein fractions, however, accompany changes in the rate of sedimentation. Fibrinogen or fibrin is relatively increased in the fast, and decreased in the slow, blood. The surface tension is relatively increased in the fast, and decreased in the slow, blood. Globulin in the fast blood is relatively high in the form of euglobulin. In the slow blood, the globulin fraction is relatively decreased. Changes in albumin are the reverse; that is, the fast blood shows diminished, and the slow increased, albumin. Thus the increase in albumin compensates for the changes in the globulin fractions.

#### DISCUSSION

JOHN A. KILLIAN: The conspicuous feature of these experiments, it seems to me, is this: When you take the red blood cells from blood that has a fast rate of sedimentation, centrifugate them off, wash them in physiologic solution of sodium chloride, and suspend them in the plasma of blood that has a slow rate of sedimentation, their speed of sedimentation is greatly increased; in other words, it is put in the range that is considered normal. On the other hand, if you take the red cells from normal blood, centrifugate them off, wash them and suspend them in the plasma of blood that has a fast rate of sedimentation, these cells sediment rapidly, so that they are then in the range that we consider to be pathologic. In the preliminary studies on the influence of centrifugating and washing the red cells with physiologic solution of sodium chloride on the rate of sedimentation the cells in their own plasma, it was observed that apparently these two processes have little effect. These experiments seemed to indicate that the factors that influence the rate of sedimentation of the red cells are in the blood plasma. However, this does not appear to be the entire explanation, for in the case of secondary anemia showing no leukocytosis and a fast rate of sedimentation of the red cells, there is a definite change in the total solids of the blood and in the red cell volume. Of course, the change in the total solids of the whole blood may be associated with a change in the red cell volume. It becomes a more and more complex problem to explain the factors that influence the rate of sedimentation, and so far, I think, the only indication we have of the nature of these factors is from this cross-sedimentation experiment. The results for surface tension are suggestive, but what they mean I do not know. In an association of two phenomena of this kind, the fast rate of sedimentation and the increased surface tension, the question arises: Which is the cause and which is the effect? I do not think we can conclude that the changes in the surface tension of the plasma are necessarily associated with the rate of sedimentation in the nature of cause and effect.

One important finding made in seventy-five analyses of blood, representing a wide range of pathologic conditions, was that the total serum protein in each case was well within normal limits. Considerable variations were noted in the fractions, particularly in globulin, as compared with the albumin. fraction. The variations

in the globulin fraction were always found in the euglobulin, and not in the pseudoglobulin, but these appeared to be always compensated for by the inverse variations in the albumin fraction.

ARTHUR F. COCA: The speakers have not mentioned the blood groups, which we are especially interested in. I wonder whether the plasma with which these cells were mixed was from persons from the same blood group.

JOHN A. KILLIAN: Yes.

#### PRECIPITATION TESTS FOR SYPHILIS. HARRY EAGLE (by invitation).

Fundamentally, all the precipitation tests now used for the diagnosis of syphilis are identical. An alcoholic extract of mammalian tissue is diluted with some sort of aqueous solution, forming a suspension of lipid particles. These have little tendency to cohere, and remain discrete when added to normal human serum. In syphilitic serum, however, they combine with a reactive substance (reagin), which forms sensitizing films of denatured protein around the individual lipid particles. When the stabilizing surface charge on these films is reduced below a critical level, as it is by dilute electrolyte, these charged films fail to repel each other, and the particles cohere on impact.

Despite this underlying identity, the tests now in use differ materially. The points of difference fall into two general categories: (1) those that affect the properties of the lipid suspension used as antigen and (2) those that affect the aggregation of the lipid-reagin compound. In the first group belong such factors as the source of the lipid, the method of purification, the method of extraction, the materials used for sensitizing the antigen, the lipid; the quantity of salt solution used to dilute and its concentration, the method of dilution, etc. To the second group belong such factors as the antigen-serum ratio, the duration of shaking, the duration and the temperature of incubation, the total volume, the method of reading, etc. These variables adequately describe every precipitation test now in use.

In general, the best of the precipitation tests are more sensitive than the best of the Wassermann technics. Their fault lies in the difficulty of reading weak positive reactions. The indefinite character of the aggregation in such cases calls for close reading, and this in turn makes for the reporting of false positive reactions. Improvement in the precipitation tests can be expected along three lines. In the first place, the optimum values of the variables listed in the foregoing paragraph can be determined on both theoretical and empirical grounds; the application of these optimum values in the precipitation test will necessarily result in greater sensitivity and more clearcut results. In the second place, new sensitizing substances will be used to replace or supplement cholesterol. The greater sensitivity of a cholesterolized antigen is due to the fact that the cholesterol forms myriads of microscopic and submicroscopic particles that adsorb the active lipid. The visible result is an increased opacity, due to the presence of many more microscopically visible particles. The coarsened dispersion facilitates aggregation; moreover, the complex lipid-cholesterol particles have an unexplained greater avidity for reagin. Since the sensitizing action of cholesterol is due solely to its physical properties, any substance with similar physical properties can be expected to have a similar sensitizing action. This has been found to be the case. The use of two other sterols, derived from corn germ and wool, as adjuncts to cholesterol, has resulted in a significant increase in the size of the aggregates formed, and thus, in the sensitivity and clarity of the results. Finally, insufficient use has been made of the centrifuge as a means of facilitating aggregation.

Eventually the precipitation phenomenon will probably supplant the fixation technic as the diagnostic criterion for syphilis, but not until some method has been found for increasing the sensitivity of precipitation tests with spinal fluid. Even the most sensitive precipitation tests now available are inferior to the Wassermann test with large quantities of spinal fluid, and until this difficulty has been successfully overcome, the Wassermann test cannot be considered, as it is by some serologists, an anachronism.

## DISCUSSION

WARD J. MACNEAL: I wish to congratulate Dr. Eagle and to express my pleasure at listening to his description, which is of course fragmentary, of the large amount of work that he has done in an attempt to put this reaction on a more precise and scientific basis than it has been. Some ten or fifteen years ago there were serologists who felt that the last word had been spoken about the serologic tests for syphilis, or, at least, that the last word ought to have been spoken. However, I think that those who have had to deal practically with the problem have not shared this opinion. The serologic tests for syphilis are of the greatest value in the handling of this important disease. I believe that a matter that is so important practically, requires continuous study, and certainly offers opportunities for obtaining more exact knowledge than we possess at the present time.

The attitude of the clinician toward laboratory work in syphilis deserves some general remarks. I recall my professor in syphilology stating thirty years ago that syphilis is one disease in which the laboratory has nothing to offer, and that it is a disease in which the clinician needs no help from the laboratory, because the clinical evidence is so clearcut and definite that a physician can recognize the disease when it exists. There has been a remarkable transformation in the attitude of the syphilologists in the last thirty years, for now, at least in this city, one finds that the case of a syphilitic patient is sometimes briefly recorded as "Wassermann + + + +" or "Kahn + + + +." There is little record of the tale of woe and practically no record of a physical examination on the part of the physician. The syphilologists of thirty years ago would probably turn over in their graves if they should see the way in which a case of syphilis is handled and the emphasis that is placed on the laboratory tests. Those of us who perform the tests have much less confidence in them than many of the clinicians who receive the reports. They seem to take that "4" as being the last word in the recognition of syphilis, and I think that the laboratory men who see the other side sometimes have great doubts. I have gone over histories and have become convinced that occasionally the patient is being treated for having a figure 4 on a sheet of paper, rather than for any form of disease that exists in him. Undoubtedly there are errors of this sort that are rather tragic for the patient.

I have been interested in following the work that Dr. Eagle has done, and I am sure that new light has been thrown on this problem by him. However, any new technical procedure has to pass the test of application to a considerable number of cases. I should like to see the statistics divided into three groups instead of two, the first group to include statistics from the application of the test to persons in whom there is no suspicion of the existence of syphilis; the second group, those from its application to persons who are known to have syphilis, but who have not been treated for this disease for a considerable period of time, and the third group, those from application of the test to syphilitic patients under treatment. Unfortunately, one is able to get a large number of data of the first and the third groups, but it is not so easy to get a large number on persons who are known to be syphilitic, but who have been without medication for a long time. Syphilitic patients under treatment are the ones, in my own observations, who have shown the greatest serologic discord. On that account, I feel that several different tests ought to be employed for syphilitic patients under treatment, so as to indicate to the clinician in charge of the case that there still remains in the blood of the patient something that makes it different from the blood of a person who has not had syphilis. Even though some of these tests are wholly incapable of detecting that difference, some of the other tests detect it. In my opinion, the blood of a syphilitic person who gives a positive reaction with any one of these tests should be regarded as showing evidence of syphilitic disease. However, there are syphilographers who do not take this view, and who are willing to believe that a patient with a negative reaction in some one test has been relieved of his infection.

ARTHUR F. COCA: I have not much to add to what Dr. MacNeal has said. I agree with him in general and in detail. When he said that in the old days the clinicians did not need the laboratory in order to make a diagnosis of syphilis, he could have added that in some laboratories the technician does not need the clinician to make a diagnosis of syphilis. He pointed out especially the seriousness of the results of the test. It is different in that respect from almost any other clinical test. It is a colloidal reaction or depends on a colloidal reaction which is notoriously uncertain, especially in the zone at the end of the titer, that is, just where we have to make our most difficult readings in the case of syphilis in which treatment has been given or in that of a weak reaction. If one should send a specimen of urine to a dozen laboratories to be tested for the presence of sugar, even one of a urine in which there is only a very small quantity of sugar, there will be hardly any difference of opinion among these laboratories as to the result. But if one sends a serum in which the precipitation is weakly positive, or the serum of a treated syphilitic patient, to different laboratories, the results will be found to be in disagreement in four of five cases. In other words, we are never sure in such specimens of any particular serologic state of the serum.

Finally, I think we ought to remember what Dr. Eagle has said about the difficulty that surrounds the proper performance of precipitation tests. We have to remember in how many laboratories these important tests are in the hands of technicians who have practically no understanding of the fundamental nature of the reactions. I should like to emphasize what Dr. MacNeal has said in one more general point, and that is that any test that is to replace the Wassermann or the precipitation test will have to be tried out on the several categories of syphilitic patients, especially the treated syphilitic patients. Unless the nature of the reaction can be changed considerably, it does not seem to me that there is much hope of there being a test devised that will give the same result on the same serum with different antigens. Take such a simple test as the agglutination test. Many years ago I heard that von Dungern, Neisser and Sachs were testing the same agglutination test, attempting to check one another's results in the final titration. They were unable to do it, although they tried many times; and last year Landsteiner in Paris met with three or four other competent serologists and attempted to titrate the same grouping serum against the same suspension of blood. The results differed as much as eight times in the titer. This inconsistency is a property of all kinds of immunologic reactions, which I have not much hope of our being able to get over.

HARRY EAGLE (closing the discussion): The question which Dr. MacNeal brought up of the so-called + + + + reaction is interesting. As you know, + + + + is supposed to mean strongly positive; + + +, + + and +, decreasing degrees of positivity, and 0, negative. Actually, the terms do not mean anything of the sort. The degree of positivity of a syphilitic serum can be expressed in the same terms as the titer of a positive Widal serum. If one takes the serum from a patient with active syphilis and does a quantitative Wassermann test by serial twofold dilutions of the serum, one finds that the serum may give a titer as high as 400; that is, diluted 400 times, the serum will still give a positive Wassermann reaction. This is rather unusual, but one gets a few with a titer of 200, and a large number with a titer of 100. In other words, + + + + does not necessarily mean a strongly positive reaction. It may mean anything from a serum containing one unit to a serum containing 400 units. And since the designation + + + + does not mean a strongly positive reaction, it should be dropped. The designations + + +, + + and + do not mean decreasing degrees of positivity. They may mean weakly positive reactions, but they may also mean a technical error in the laboratory. It has been recommended by the League of Nations, and the recommendation should be adopted, that in reporting the results the terms "positive" and "negative" or "doubtful" be used. Indefinite aggregation in the precipitation test also calls for a doubtful report, and any such doubtful report calls for a repeated test.

As Dr. MacNeal and Dr. Coca said, it is tragic to label a patient syphilitic because of a single positive reaction. I had rather report fifty false negative reactions than one false positive reaction. I put the ratio even higher, because if the patient learns of the report, no number of negative reactions is going to erase the suspicion that he may have syphilis.

Regarding the incidence of a positive Wassermann reaction in different types of syphilis, we have been much struck by the fact that the incidence of positive serologic reports is much higher than the books give it. In the literature, the incidence of a positive serologic finding ranges from 30 to 100 per cent in the various types of syphilis. In going over our reports for the last fifteen years, we found that the average for all types of syphilis by the icebox Wassermann technic is around 90 per cent. Finally, I think that the precipitation tests can be made to be reliable, but not until the weak positive reactions can be clarified. Until the difference between a weak positive and a negative reaction is unmistakable, all reports of weak positive reactions should be suspected. And it is the laboratory worker himself who should be the most conservative in the interpretation of results.

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## PATHOLOGICAL SOCIETY OF PHILADELPHIA

*Meeting of Dec. 10, 1931*

BALDUIN LUCKÉ, *President, in the Chair*

A FOUR-CUSPED PULMONARY VALVE. W. T. READ, JR.

A white woman, aged 31, who died suddenly eleven days following cholecystectomy, had given no evidence in history or in physical examination of cardiac disease, except for slight pretibial edema. At autopsy, marked dilatation of the right side of the heart was found, with a four-cusped pulmonary valve. A brief report of the number of such cases in the literature was given.

BILIARY CIRRHOSIS WITH ATRESIA OF THE EXTERNAL BILE DUCTS AND ABSENCE OF THE SPLEEN. W. T. READ, JR.

A white female infant was studied until her death at the age of 10 months. Jaundice, light-colored stools and dark urine were noted from the fifth week of life on. These symptoms fluctuated but never disappeared. Terminal ascites developed. During life, the infant had bilateral otitis media. At autopsy, a markedly cirrhotic liver of the biliary type was found. The common duct could not be identified, and fibrous remnants of the cystic and hepatic ducts were all that could be demonstrated. The gallbladder was small and fibrous, and contained no bile. A thorough examination of the abdominal cavity revealed no spleen. A chain of nodules was found that, on gross examination, were thought to be, possibly, small splenic nodules. Histologically, these proved to be hyperplastic lymph nodes.

HISTOGENESIS OF ATROPHIC CIRRHOSIS. V. H. MOON.

This article will appear in full in the ARCHIVES.

OBSERVATIONS OF LYMPHATIC CAPILLARIES STUDIED MICROSCOPICALLY IN THE LIVING MAMMAL. E. R. CLARK and E. L. CLARK.

Moving pictures were shown of lymphatic vessels growing in the transparent chamber in the rabbit's ear.

## Book Reviews

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**Intracranial Pyogenic Diseases: A Pathological and Clinical Study of the Pathways of Infection from the Face, the Nasal and Paranasal Air-Cavities.** By A. Logan Turner, M.D., LL.D., F.R.S.E., Consulting Surgeon, Ear and Throat Department, Royal Infirmary of Edinburgh, and F. Esmond Reynolds, M.D., D.T.M. & H., M.R.C.P., Superintendent of the Laboratory of the Scottish Asylums' Pathological Scheme. Cloth. Price, 12/6. Pp. 271, with 82 illustrations. Edinburgh: Oliver & Boyd, 1931.

Fatal intracranial infection secondary to an inflammatory focus on the face or in the nasal and paranasal air cavities is not uncommon. Experimental and microscopic investigations have been carried out on the pathways of infection in tuberculosis and epidemic meningitis and in the group of infections of the central nervous system attributed to the filtrable viruses, but in the literature there are few records of a systematic and comprehensive microscopic investigation of the pathways of pyogenic infection of the brain and its membranes from extracranial septic foci. This scholarly treatise is the result of nine years of investigation of this subject. The authors realize the advantage of a close and sympathetic cooperation between the pathologist and the clinician, and throughout the work such a cooperation is evident.

The material for this investigation was obtained from a series of fatal cases of intracranial infection, secondary to an inflammatory focus on the face and in the nasal and paranasal air cavities, and in twenty of the cases an attempt was made to demonstrate by microscopic preparations the actual path by which the infection reached the intracranial structures. In addition to the twenty cases examined in this detailed manner, there is a further group of thirty-five cases, nine of which were studied clinically and twenty-six both clinically and post mortem. The primary septic focus was situated on the face in four cases, in the paranasal air cavities in forty-five cases, in the fauces in one case and in the middle ear cleft in five cases. The secondary involvement included forty-nine cases of intracranial complications and six cases of general blood infection in which no localized intracranial infection was present or at any rate demonstrable at autopsy.

The arrangement and presentation of the material are excellent. After a review of the literature on the subject, the methods employed in the investigation and a tabular statement of certain main facts concerning the fifty-five cases investigated, a general consideration of the pathways of infection of the intracranial structures is presented. The anatomic details necessary for the correct interpretation of the cases are clear and concise. The diagrams, illustrations, photographs and photomicrographs are well chosen. Twenty-two of the microscopic illustrations are in colors. Each of these illustrations is accompanied by a full page explanatory diagram. The cases investigated are grouped to illustrate the common pathways of infection of the intracranial contents. An account of the history of the case, the progress of the infection and the physical and postmortem observations, a brief summary of the bacteriology and a detailed account of the microscopic examination of the tissues in an effort to trace the pathway of the infection are given. The comments on the results of the investigation in each case are logical and conservative. The correlation of the clinical signs with the underlying anatomic change is emphasized.

An analysis of the pathways of infection from the various primary foci shows that the venous blood stream carried the infection in nineteen cases—thirteen of these were of cavernous sinus thrombosis and of general blood infection; that direct extension through the bone was responsible in twenty cases and a combina-

tion of direct extension and blood stream infection in eight and that infection was transmitted along the olfactory perineural sheaths in four. In four cases, none of which was examined microscopically, the pathway could not be determined.

From the evidence presented the authors emphasize that when infection passes by the blood stream from an inflammatory focus on the face or in the nasal or paranasal air cavities to the intracranial structures, the initial infection of cutaneous or mucosal veins gives rise, as a rule, to infective thrombosis of the cavernous sinus. The pathway of infection in thirteen of the cases was entirely by way of its afferent or efferent venous channels from the face, pharynx, air cavities and middle ear cleft. In eight of the cases the preliminary stage of the infective process was by direct extension through the bone, followed by further spread to the sinus along the osseous veins. In the latter group, the primary focus was in the frontal, ethmoidal or sphenoidal air cavities. Infection of an osseous vein in the wall of the paranasal air cavity or in any of the bones forming the cranial box may give rise to purulent leptomeningitis.

When the process advances by direct extension through the bone and dura, diffuse leptomeningitis is the usual result. In these cases various stages mark the progress of the infective process; these are osteomyelitis with or without an extradural abscess, then pachymeningitis, subdural infection and leptomeningitis. When extension of the inflammatory process passes along the olfactory perineural sheaths, leptomeningitis is set up; at first it is limited to the cribiform plate, but later becomes generalized.

Septicemia and pyemia may develop in cases of osteomyelitis of the cranial bones without intercurrent infection of any of the blood sinuses. Six cases of this type are recorded. In all of them the primary focus was in one of the paranasal air cavities. The complication may arise immediately following an operation on the cavities or spontaneously in the natural course of the disease in these cavities.

No attempt has been made by the authors to investigate the bacteriology of the cases in the series. This is unfortunate. However, certain data have been collected from the routine bacteriologic examination in forty of the cases, and these results are summarized in one chapter. There is an extensive bibliography. No discussion of the treatment is offered. The treatment depends to a great extent on a knowledge of the process in each case and on a careful study of the changes arising in consequence of the particular pathway pursued by the infection. This book will be of interest to all who are concerned with such conditions.

**Tumours of the Breast: Their Pathology, Symptoms, Diagnosis and Treatment.** By Sir G. Lenthal Cheate, K.C.B., C.V.O., F.R.C.S., Consulting Surgeon and Emeritus Lecturer on Surgery, King's College Hospital, London; Late Surgeon to and Lecturer on Surgery at King's College Hospital, London; Walker Prizeman, 1926-1930; and Max Cutler, B.Sc., M.D., Director of Tumour Clinic, Michael Reese Hospital, Chicago; Late Clinical Fellow, Memorial Hospital, New York; Director of Research Division of Cancer Department of Hospitals, New York; Attending Radiation Therapist, New York City Cancer Institute. Price, \$12. Pp. 596, with 18 colored plates and 468 other illustrations. Philadelphia: J. B. Lippincott Company.

In this comprehensive and competent work the reader will find a veritable mine of information regarding every department of knowledge of the physiology and pathology of the breast, and the specialist will encounter a detailed and expert discussion of all those practical and theoretical problems of diagnosis and treatment which render tumors of the breast a difficult medical specialty. Throughout these pages one detects the broad experience and philosophical attitude of the senior author, aided by the industry and discernment of his younger collaborator. If there is any fault to be found in the work as a whole, it lies in the richness of material available, the freedom with which it is presented and the elaborate discussions and analyses with which the central questions at issue are pursued.

It is no treatise for the beginner, but the specialist in many lines who brings adequate familiarity with him will delight and profit in following the authors to the full limit in their presentations. This task is rendered comparatively easy by a profusion of photographs and colored plates, which present the morphologic side, probably more fully than has ever before been attempted. In this field the great value of sections of the whole breast, by the methods developed by the senior author, is constantly apparent, and the student should not fail to make full use of these impressive reproductions. With the exception of the opening chapters, all the material in this work is new and represents the original observations of the authors.

After an adequate review of the anatomy and embryology of the breast, there follows a valuable summary of data on the interrelations of the breast with the uterus and ovaries through the action of hormones, and some consideration of the use of ovarian extracts in the treatment for mammary diseases.

The various forms of chronic mastitis are regarded as physiologic and functional disturbances, and not as inflammatory. The fibrous form is designated as "desquamative epithelial hyperplasia," and the term "mazoplasia" is introduced. Cystic mastitis is called "cystiphorous desquamative epithelial hyperplasia," and is regarded as an important antecedent of carcinoma. It is emphasized that the essential carcinomatous process begins before there is actual invasion. About 20 per cent of all mammary cancers are found to begin in the lesions of the cystiphorous state. Many will regard this proportion as too low, and inquire how the other 80 per cent begin. The numerous precancerous phases of this condition are fully traced.

Carcinoma of the breast is presented fully from every angle, including histologic stages, grades of malignancy and radiosensitivity, modes of extension, local and general; methods of diagnosis by palpation, transillumination, aspiration and biopsy and methods of treatment by surgery and radiation. Some readers may not be entirely satisfied with the prominence given to the various gross anatomic phases of mammary cancer. The facts are there, but they do not stand out. If this is a fault, it is more than balanced by effective reference to many less common benign and malignant conditions in the breast which are often dismissed too lightly by most writers.

Paget's disease occupies a prominent position in the work, elaborately illustrated and competently discussed from the detailed study of seventeen cases. The view is maintained and adequately supported that Paget's disease is a primary carcinoma of the skin of the nipple, often involving the epithelium of the terminal ducts and frequently, but not always, associated with single or multiple foci of cancer in the deeper duct system. Here and elsewhere the authors acknowledge the aid of the fine technical skill and pathologic interpretation of Dr. J. D. Ludford.

An important section relates to the radiation treatment for mammary cancer, both operable and inoperable. The data on the various phases of this question are rather fully presented and conservatively discussed. While some remarkable results of radiation treatment by various methods are reported, the authors refrain from expressing any definite recommendations and await further progress in this experimental field. A well chosen bibliography follows each chapter.

The publication of a highly specialized work of this character, reflecting the mature experience of many years of study and observation with a large material, emphasizes anew the great complexity and difficulty of the problems of mammary tumors. It explains and justifies the tendency to regard mammary cancer as a field demanding the comprehensive knowledge and wide experience of the specialist, and warns against the assumption that these diseases may be competently handled as a side issue in the work of the general surgeon and radiologist. The authors are congratulated on having achieved this object, among others, by preparing a comprehensive work of permanent value and conveying an impressive message on this most important subject.



**Grundriss der Entwicklung der Menschen.** By Alfred Fischel. Paper. Price, 11 marks. Pp. 141, with 117 illustrations, in part colored. Berlin: Julius Springer, 1931.

Those facts that every physician should know regarding the development of the human embryo have been selected by Professor Fischel from his large textbook of human embryology and are presented in the "Grundriss" in a greatly condensed and convenient form. The demand for this kind of book is large and arises not only from the needs of the medical profession but also from the requirements of that large group of readers composed of premedical students who have to pass examinations. Several books of similar scope and character have appeared in the English language during the past few years. Professor Fischel, however, has attained a conciseness and clarity that most of the books in English lack, and to those who read German easily his book will have a strong appeal.

It is not an easy task to present, in so few pages, a complicated phenomenon, like the development of the egg and the formation of the organs of the embryo. The success that marks Professor Fischel's effort is in part to be accounted for by the fact that his pen was still moist from the writing of his "Lehrbuch," in which his conceptions and interpretations had been clarified and put down in orderly fashion. It is, of course, this larger work that is the important one and that will always redound to his credit. That such a superb product should evolve from amidst the profound disturbances of the World War is an example of that marvelous attribute of scholarship, the ability of detaching itself from surrounding political and social conditions. It will be remembered that Plato wrote his "Republic" during those same days when the city-states of Greece were blindly tearing each other apart in their bitter political rivalry, and the Academy carried on and produced students of the quality of Aristotle when the annihilation of the Athenian Empire seemed to have sealed the doom of all that was noblest and best in Greek life.

**Association française pour l'étude du cancer. Atlas du cancer. Neuvième et dixième fascicules. Les tumeurs des centres nerveux et des nerfs périphériques.** By Gustave Roussy and Charles Oberling, from the Foundation of Henri de Rothschild. Paper. Price, 80 francs. Paris: Félix Alcan, 1931.

This beautiful atlas, published by the generosity of Dr. Henri de Rothschild, has been appearing in successive fascicles. The present ninth and tenth fascicles deal with tumors of the nervous system. The illustrations are beautifully clear, as they have been in the other parts of the atlas. It is unfortunate, however, that at present there is a period of anarchy in the terminology of cerebral tumors, which is apt to confuse the uninitiated, although the authors give synonyms for the names they use. It is at any rate evident that the same types of tumors can be traced through all the recent publications concerning gliomas, whatever the terminology used. One has a little difficulty in understanding why the authors have changed oligodendroglioma to "oligodendrocytome." There is also no justification for calling a spongioblastoma an "oligodendrocytome à cellules fusiformes"; the authors offer no proof of any kind of the oligodendroglial nature of the cells of this tumor. The tumor labeled "astrocytome pseudo-papillaire" is the glioma that Bailey has described with Bucy under the name of astroblastoma. The tumor in plate 8, figure *D*, looks much like a tumor of Rathke's pouch. Nobody seems to fancy the term medulloblastoma, which Bailey introduced for the malignant cellular tumor of the cerebellum of children, but "neurospongiome" is no improvement, because it does not take account of the neuroblasts that differentiate in these tumors. With the exceptions noted, the atlas should prove useful to pathologists, especially in French-speaking countries. The histologic atlas on gliomas by Percival Bailey (*ARCH. PATH.* 4:871, 1927) covers the same field.

**Annals of the Pickett-Thomson Research Laboratory. Volume 6. The Pathogenic Streptococci. The Role of the Streptococci in Scarlet Fever.** By David Thomson, O.B.E., M.B., Ch.B. (Edin.), D.P.H. (Camb.), Hon. Director, Pickett-Thomson Research Laboratory, St. Paul's Hospital, London, and Robert Thomson, M.B., Ch.B. (Edin.), Pathologist to the Pickett-Thomson Research Laboratory. Price, \$10. Pp. 470. Baltimore: Williams & Wilkins Company, 1930.

The original plan to devote this volume to summarizing the knowledge about streptococci in erysipelas, skin diseases and scarlet fever had to be abandoned because the material on scarlet fever alone was found to require the whole volume. The succeeding volume of the Annals deals with the streptococci in erysipelas, skin diseases and measles. Like other volumes of the Annals, the volume on streptococci in scarlet fever represents an enormous amount of work. Some 1,400 papers have been abstracted and indexed. "The research work carried out on scarlet fever during the past five years is greater than the whole of the total previous researches on the subject. This great increase of research on scarlet fever in recent years is largely due to the discovery of the Dick toxin and the Dick test, which has led to new methods of investigation." The authors conclude that scarlet fever is caused by a specific streptococcus which, they say, may be justly called *Streptococcus scarlatinae*. The growing knowledge of the differences between the various pathogenic streptococci is summarized. The index has been prepared with special care in order to make it as helpful as possible. It will save workers much time and effort in hunting for references. As an abstract and catalogue of the literature on streptococci in relation to scarlet fever the volume stands without a rival.

**Osler and Other Papers.** By William Sydney Thayer, M.D., LL.D., Dr. Hon., ScD., F.R.C.P. Ire.Hon.; Professor Emeritus of Medicine at the Johns Hopkins University. Price, \$3.50. Pp. 386. Baltimore: Johns Hopkins Press, 1931.

This book includes twenty-four addresses and papers "delivered or published at intervals during an active professional and university life, comments, for the most part, on men and things medical." The first five chapters are devoted to Osler, with whom the author was associated closely during his period in Baltimore. These chapters give vivid reminiscences of the great master and happy illustrations of his character and influence. There are also brief but stimulating accounts of the life and work of Bright, Laennec, Pasteur and Fitz, as well as tributes to Howland and Welch. Other chapters include: the problems of medicine, teaching and practice, scholarship in medicine, the university and medicine and the duties and problems of the physician. The dominant note in all the chapters is the intense devotion of the author to high ideals. Idealism coupled with an accomplished pen gives the writing charm and power. It is a delightful volume.

## Books Received

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TUMOURS OF THE BREAST: THEIR PATHOLOGY, SYMPTOMS, DIAGNOSIS AND TREATMENT. By Sir G. Lenthal Cheatle, K.C.B., C.V.O., F.R.C.S., Consulting Surgeon and Emeritus Lecturer on Surgery, King's College Hospital, London; Late Surgeon to and Lecturer on Surgery at King's College Hospital, London; Walker Prizeman, 1926-1930; and Max Cutler, B.Sc., M.D., Director of Tumour Clinic, Michael Reese Hospital, Chicago; Late Clinical Fellow, Memorial Hospital, New York; Director of Research Division of Cancer Department of Hospitals, New York City; Attending Radiation Therapist, New York City Cancer Institute. Price, \$12. Pp. 596, with 18 colored plates and 468 other illustrations. Philadelphia: J. B. Lippincott Company.

ROENTGENOLOGIC STUDIES OF EGYPTIAN AND PERUVIAN MUMMIES. By Roy L. Moodie, Paleopathologist to the Wellcome Historical Museum, London. Berthold Laufer, Curator of Anthropology, Editor. Anthropology Memoirs, Field Museum of Natural History Founded by Marshall Field, 1893. Volume 3. Paper. Price, \$5. Pp. 66, with 76 plates. Chicago: Field Museum of Natural History, 1931.

THE INTERVERTEBRAL DISCS: OBSERVATIONS ON THEIR NORMAL AND MORBID ANATOMY IN RELATION TO CERTAIN SPINAL DEFORMITIES. By Ormond A. Beadle. Medical Research Council, Special Report Series, No. 161. Price, 2 shillings, net. Pp. 179. London: His Majesty's Stationery Office, 1931.

## THE SECONDARY NODULES OF LYMPH NODES

THEIR RELATION TO CHRONIC INFLAMMATORY PROCESSES \*

RALPH ENGLISH MILLER, M.D.

HANOVER, N. H.

The question of the significance of the secondary nodules of lymphatic tissue, which was considered settled by Flemming's<sup>1</sup> work (1885), was again opened by Hellman<sup>2</sup> in 1919. Since that time many workers have contributed to a solution of the problem. The discussion has concerned several apparently opposed facts. Flemming's contention that the secondary nodules were physiologically the chief sites of the regeneration of lymphocytes was supported by the single finding that there were more mitotic figures in the secondary nodules of the lymph nodes examined than in the tissues surrounding the nodules.

Although no serious objection to Flemming's theory was raised in the literature until Hellman's work appeared, there were disclosed in the interim several facts that could not be explained with entire satisfaction by that theory. The absence of germinal centers in fetal life was established. It was recognized that germinal centers are not present in the involved tissues in lymphosarcoma and lymphatic leukemia. Careful cytologic studies revealed that mitotic figures were as abundant in the lymph cords and interfollicular portions as in the secondary nodules, and it was recognized that Flemming's "tingible Körper" were disintegrating cells.

Hellman's proposition that the secondary nodules are the anatomic evidence of work performed by the lymph node in response to lymph-borne or blood-borne foreign material has received the support of many investigators. Other workers have reported their findings in support of Flemming's theory, but none has offered an explanation of the absence of secondary nodules in the fetus and in the lymphatic neoplasias.

Recently many writers, taking the middle ground, have expressed the belief that the secondary nodules represent both the "reaction centers" of Hellman and the "germ centers" of Flemming. There is in the literature considerable confusion, owing on the one hand to a lack of uniform terminology and on the other to a failure on the part of

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\* Submitted for publication, Aug. 27, 1931.

1. Flemming, W.: *Arch. f. mikr. Anat.* **24**:50, 1885.

2. Hellman, T. J.: *Upsala läkaref. förh.* **24**:57, 217 and 283, 1919.

some authors to appreciate the marked differences between fresh animal material and human postmortem material.

An investigation of the problem was undertaken on the suggestion of Dr. H. E. Robertson, who has long believed that Flemming's theory is inadequate. During the course of this investigation it became apparent that certain types of chronic inflammation are frequently associated with a hyperplasia of the regional lymph nodes. It was thought that some light might be thrown on the question of the nature of the secondary nodules if the constancy of the relationship of the lesions and the lymphoid hyperplasia was determined.

#### REVIEW OF THE LITERATURE

The secondary nodules of lymphatic tissue were first mentioned by Brücke,<sup>3</sup> who in 1850 noted cloudy-white central spots (zentralen Flecke) in the peripheral nodules of some lymph nodes and more frequently in the nodules of Peyer's patches. Brücke<sup>4</sup> noted that the afferent lymphatics of mesenteric lymph nodes of dogs fed on a diet low in fat contained clear fluid, and that the efferent lymph vessels of the same nodes contained cloudy fluid. He determined that the cloudiness of the lymph was due to the presence of lymphocytes and postulated that the lymphocytes were formed in the lymph nodes. In the medullary cords and cortical nodules, he noted the "various stages of lymphocyte formation," and he therefore designated these portions of the lymph node "Keimlager."

His,<sup>5</sup> in his classic work on lymph nodes, described clear, round areas in the cortical nodules of lymph nodes of the ox and in the nodules of Peyer's patches of the rabbit. He called these clear areas "vacuoles" and noted that their blood supply was capillary only.

Hansen<sup>6</sup> supported the observations of His, and added that there were many nodes in which no "vacuoles" could be found, and that the "vacuoles" were demonstrable only after birth. He considered the light central areas as due to an atrophy brought about by a proliferation of the lymphocytes and a consequent interference with the blood supply. He found no microscopic evidence to account for the increased number of lymphocytes in the efferent lymph vessels.

Frey<sup>7</sup> merely stated that within the large cortical follicles of the lymph nodes of oxen are other clear follicles, which His had called

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3. Brücke, Ernst: Ueber den Bau und die physiologische Bedeutung der Peyer'schen Drüsen, *Wissenschaftliche Denkschriften der wiener Akademie*, 1850, vol. 2, p. 21.

4. Brücke, Ernst: *Wien. Akad. Mar. Nat. Kl.* **6**:99, 1854.

5. His, W.: *Ztschr. f. wissenschaft.* **11**:65 and 416, 1861.

6. Hansen, G. A.: *Virchows Arch. f. path. Anat.* **56**:280, 1872.

7. Frey, H.: *Handbuch der Histologie und Histochemie des Menschen*, ed. 4, Leipzig, W. Engelmann, 1874, p. 421.

“vacuoles.” Toldt<sup>8</sup> also gave these clear central areas scant mention, stating that they were seen in “some lymph follicles.” Toldt used the term lymph node in preference to that of lymph gland.

Flemming<sup>1</sup> established an anatomic and physiologic interpretation of the cortical nodules of lymph nodes that was to remain unchallenged in the literature for many years. He reviewed the literature critically, deploring the continued use of the term lymph gland. He supported the use of the term node instead of the term gland, and the use of the term nodule instead of the term follicle, pointing out that the term follicle was not applicable to a solid structure. He adopted Brücke’s designation of the combined medullary cords and cortical nodules as “Keimlager.” Flemming considered the “zentralen Flecke” of Brücke and the “Vacuolen” of His as identical with the structures that he called anatomically “secondary nodules” and physiologically “germinal centers” (Keimcentren). Flemming examined three lymph nodes from two oxen, the pancreas asellii and Peyer’s patches from two rabbits and the lingual tonsil of a man. He found mitotic figures in the medullary cords and in the lymph sinuses of the lymph nodes, but most plentifully in the light centers of the cortical nodules and Peyer’s patches. It was because of the abundance of mitotic figures in these nodules that he called them “germinal centers.” He defined the germinal center as a light area with a sharply defined border of compressed reticulum packed with lymphocytes, which in turn was surrounded by an area of less densely packed lymphocytes. The center he described as consisting of cells with abundant cytoplasm and therefore with nuclei widely separated. Among these cells, he noted few lymphocytes and many large cells containing deeply staining bodies, which he designated “tingible Körper.” Because some of the mitotic figures were apparently in reticular cells, he considered the possibility that the reticulum might be the “mother substance” from which the lymphocytes were formed. In the lingual tonsil, he found some nodules without mitotic figures, and he explained this on the basis that the specimen was fixed one hour post mortem. He expressed the belief that cells in the process of mitosis might complete their division after death, but that new mitoses would not be initiated. He recognized that the secondary nodules varied in size and concluded that they were labile structures, increasing and decreasing in activity periodically. To explain the scarcity of lymphocytes in the secondary nodule and their accumulation about its periphery, he postulated a centrifugal pressure mechanism that forced the daughter cells to the periphery of the nodule. Flemming had previously noted secondary nodules in spleens, and he concluded unequivocally that the secondary nodules of the lymph nodes,

8. Toldt: *Lehrbuch der Gewebelehre*, ed. 2, Stuttgart, F. Enke, 1884.

spleen and alimentary tract were the principal places for the formation of lymphocytes. He believed that the fluctuation of the size of the centers was dependent on physiologic stimuli.

Several of Flemming's students carried on studies on other lymphatic structures. Drews studied tonsils of various animals; Moibus and Heilbrunn, spleens of animals and man. These workers supported the findings of Flemming, and Paulsen,<sup>9</sup> another of Flemming's students, studied "hyperplastic" lymphatic structures to determine if the regeneration of lymphocytes was the same in these as in normal lymphatic tissue. Paulsen used a lymph node, a "so-called rheumatic bubo," from a 48 year old man, the "hypertrophic" pharyngeal tonsils of an 18 year old youth and several "hypertrophic" faucial tonsils from persons from 8 to 18 years of age. He described an increase in the number and size of the secondary nodules and an increase in the number of mitotic figures in these nodules. He concluded that regeneration of lymphocytes followed the same plan in these structures as in the normal. He also found the "tingible Körper" to be most abundant in tonsils and postulated a relationship between their number and the number of mitotic figures.

Baumgarten<sup>10</sup> and Ribbert<sup>11</sup> had demonstrated the abundance, and Downey and Weidenreich<sup>12</sup> the preponderance, of mitotic figures outside the secondary nodules; Bunting<sup>13</sup> had expressed the belief that the secondary nodules were the sites of regeneration of lymphatic tissue as a whole; Naegeli<sup>14</sup> had found large "germ centers" in many infectious diseases coincidentally with a lymphopenia and expressed the belief that this was inconsistent with the "germ centers" being a site for production of lymphocytes; Marchand<sup>15</sup> showed some skepticism regarding Flemming's theory, but it remained for Hellman<sup>16</sup> to summarize the accumulated evidence and offer observations of his own in support of a theory opposed to that of Flemming.

Hellman's observations<sup>17</sup> that "germinal centers" are not found in normal fetuses has abundant support in the literature. Gulland<sup>18</sup> examined fetuses of man, rabbit, sheep and guinea-pig and found no

9. Paulsen, E.: *Arch. f. mikr. Anat.* **24**:345, 1885.

10. Baumgarten, P.: *Ztschr. f. klin. Med.* **9**:93, 1885; **10**:24, 1886.

11. Ribbert: *Beitr. z. path. Anat. u. z. allg. Path.* **6**:187, 1889.

12. Downey, Hal; and Weidenreich, F.: *Arch. f. mikr. Anat.* **80**:306, 1912.

13. Bunting, T. L.: *J. Anat. & Physiol.* **39**:55 and 178, 1905.

14. Naegeli, O.: *Blutkrankheiten und Blutdiagnostie*, Berlin, W. de Gruyter & Company, 1912, p. 279.

15. Marchand, F.: *Verhandl. d. deutsch. path. Gesellsch.* **16**:5, 1913.

16. Hellman, T. J.: *Upsala läkaref. förh.* **24**:283, 1919; *Beitr. z. path. Anat. u. z. allg. Path.* **68**:333, 1921.

17. Hellman (footnote 2, second reference; footnote 16, second reference).

18. Gulland, G. L.: *J. Path. & Bact.* **2**:447, 1894.

"germinal centers." Baum<sup>19</sup> reported that he and his students had not been able to find "germinal centers" in horse, pig or dog before the fifth day of life nor in the cow before the eleventh day. Gundobin<sup>20</sup> found, on investigating lymph nodes of 50 new-born infants and 100 children from 1 to 2 years of age, that the first "germinal centers" appeared at the age of 2 months, and that typical, well formed "germinal centers" appeared at 2 years of age. Jolly and Rosello<sup>21</sup> found that the spleen of rats did not contain "germinal centers" up to 3 weeks of age, and that the centers did not reach full development until 2 months of age. Goslar,<sup>22</sup> on investigating 34 human tonsils from still-born infants, living infants and adults, concluded that "germinal centers appear relatively late in extra-uterine life." Parodi,<sup>23</sup> in a study of the lymph nodes of 66 human beings varying from 35 cm. fetuses to adults of 85 years found no "germinal centers" in the fetuses and rarely in persons more than 30 years of age. Ehrich<sup>24</sup> studied chiefly the axillary lymph nodes of 48 fetuses and new-born infants, varying from 14 to 40 weeks in utero, and found "secondary nodules" in a single case only. This was in the spleen of a 34 cm. embryo the mother of which had died of pneumonia. Hellman<sup>25</sup> was unable to find secondary nodules in the tonsils of rabbits before birth, and he was able to find in the literature<sup>26</sup> but a single report of an instance of "germinal centers" in a fetus. This was a case reported by Margerstedt<sup>27</sup> in which there were "germinal centers" in an inflamed appendix. Hellman reported a case of his own in which there were well formed "germinal centers" in the spleen of a 1 day old syphilitic infant. Collins<sup>28</sup> found no secondary nodules in an examination of 50 fetal appendices.

It appears to be well established, that, except when definite abnormality can be demonstrated, lymphoid tissue in the fetus and the new-born infant is without secondary nodules. But Bunting<sup>29</sup> stated that

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19. Baum, H., in Ellenberger, W.: *Handbuch der vergleichenden mikroskopischer Anatomie der Haustiere*, Berlin, Wilhelm Paul Parey, 1906, vol. 2, p. 105.

20. Gundobin, N. P.: *Jahrb. f. Kinderh.* **64**:529, 1906.

21. Jolly, J., and Rosello, H.: *Compt. rend. Soc. de biol.* **66**:40, 1909.

22. Goslar, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **56**:405, 1913.

23. Parodi, U.: *Haematologica* **8**:1, 1927.

24. Ehrich, W.: *Am. J. Anat.* **43**:385, 1929.

25. Hellman (footnote 2, first reference).

26. Hellman (footnote 16, second reference).

27. Margerstedt: *Untersuchungen zur normalen und pathologischen Anatomie des Wurmfortsatzes*, Diss., Berlin, 1908, cited by Hellman.

28. Collins, D. C.: Personal communication to the author; data to be published.

29. Bunting, C. H.: *Diseases of the Lymph Glands*, Nelson's Loose Leaf Living Medicine, New York, T. Nelson & Sons, 1921, vol. 3, p. 347.



Sabin found "germinal centers" in embryos. Sabin,<sup>30</sup> in describing the 13 cm. stage of a pig embryo, said, "The second point in advance is the formation of the germinal center. Within the follicle . . . there are small clumps of cells, *definitely lymphocytes*, heaped around a capillary tuft. In the entire node at this stage there are eight of these 'germinal centers'." The structure is obviously not a "germinal center" as described by Flemming. Nor is it a germinal center in the sense in which the term is used generally in the literature. Ehrich<sup>31</sup> pointed out that if the term germinal center is to be used, it should be limited in its application to the structure originally described by Flemming.

Hellman<sup>26</sup> pointed out that secondary nodules are found in many pathologic conditions. He<sup>25</sup> had found, in the course of his studies of the lymph nodes in carcinoma and tuberculosis, that lymphatic hyperplasia was frequent in chronic infections and toxic conditions, and that the secondary nodules played a considerable part in the hyperplasia. Saltzman<sup>32</sup> found an increase in the size and number of "germ-centers" in the gastric mucosa in carcinoma of the stomach. Matko<sup>33</sup> found the "germ-centers" increased in size and number following subcutaneous and intravenous injections of typhoid vaccine. The change was most marked in the regional nodes following subcutaneous injections. Stschastnyi<sup>34</sup> produced similar changes by the injection of proteins. Many workers (Bizzozero,<sup>35</sup> Welch and Flexner,<sup>36</sup> Oertel,<sup>37</sup> Barbacci<sup>38</sup> and others) demonstrated the necrotic type of secondary nodule in diphtheria, and Barbacci produced the same changes in lymph nodes of guinea-pigs by subcutaneous injection of diphtheria toxin.

Hellman<sup>26</sup> further offered in support of his working hypothesis (that the secondary nodules are "reaction centers") his observations concerning the lack of correlation between the blood lymphocyte count and the state of the secondary nodules in rabbits. In 1914, he had found by weight two peaks in the developmental curve of the lymphatic tissue of "normal rabbits."<sup>25</sup> One peak, that at puberty, corresponded with the period at which the rabbit normally has the greatest number

30. Sabin, F. R.: Am. J. Anat. **4**:355, 1905.

31. Ehrich, W.: Am. J. Anat. **43**:347, 1929.

32. Saltzman: Arb. a. d. path. Inst. zu Helsingfors **1**:335, 1913.

33. Matko, J.: Ztschr. f. exper. Path. u. Therap. **19**:437, 1918.

34. Stschastnyi, S. M.: Beitr. z. path. Anat. u. z. allg. Path. **38**:456, 1905.

35. Bizzozero, Giulio: Medezinisches Jahrbuch, 1876, p. 203.

36. Welch, W. H., and Flexner, Simon: Bull. Johns Hopkins Hosp., **1-2**:107, 1891.

37. Oertel, M. J.: Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung, Leipzig, F. C. W. Vogel, 1887; cited by Hellman.

38. Barbacci, O.: Zentralbl. f. allg. Path. u. path. Anat. **7**:321, 1896.

of lymphocytes in the blood.<sup>39</sup> This increase in weight of the lymphatic tissue was due to a hyperplasia of the lymphatic tissue in general, whereas the peak at the age of 10 months was due to an increase only in the peripheral lymphatic tissue. In another work,<sup>25</sup> he had found that rabbits' tonsils reached their greatest development at the age of 10 months, and that their hypertrophy was due to a hyperplasia of the secondary nodules. He argued that the peripheral location of the secondary nodules in the afferent lymph path indicated their significant relationship to incoming material, and that the location of the most highly developed nodules immediately under the epithelium of the respiratory and alimentary tracts argued for the same conclusion.

Hellman laid considerable stress on the sharp demarcation between the secondary nodule and the narrow lymphocyte border, arguing that the persistence of the sharp demarcation and the border in the presence of necrosis of the center (as in diphtheria) indicated that the structure could have nothing to do with the production of lymphocytes. He further stressed his finding that the absolute weight of the dark borders of the nodules (as determined in rabbits' tonsils<sup>40</sup>) always remained the same, regardless of the size of the secondary nodule.

The necessity for distinguishing various types of secondary nodules structurally and functionally was pointed out by Groll and Krampf.<sup>41</sup> They described 6 types of secondary nodule: the "germ center" of Flemming, the epithelioid type first described by Stilling,<sup>42</sup> the hemorrhagic type, the necrotic type (diphtheria), the solid type (consisting of lymphocytes only) and the hyaline type. Of the 300 spleens that they studied, 36 were from "normal" persons. In these 36 spleens 4 types of center were found: the "germ center" and the epithelioid, the hyaline and the solid types up to 25 years of age; thereafter, only the epithelioid and the hyaline types. They concluded that the epithelioid and hyaline types of center represent involutionary changes and are evidence of decreased production of lymphocytes in the spleen. The authors concluded from the examination of the spleens from human beings dying of infectious diseases, pyemia, tuberculosis, cachexia, carcinoma and sarcoma that lymphocyte building in the spleen decreases with age and in these conditions by a failure of the "germ centers." They found no secondary nodules of the active type in the cases of tuberculosis, cachexia, carcinoma or sarcoma. In but 10 per cent of the cases of infections, sepsis and pyemia were there secondary nodules of the active type, whereas 66.66 per cent of the centers in the

39. Lindberg, G.: *Folia haemat.* **9**:64, 1910.

40. Hellman (footnote 2, second reference).

41. Groll, H., and Krampf, F.: *Centralbl. f. allg. Path. u. path. Anat.* **31**:145, 1920.

42. Stilling, H.: *Virchows Arch. f. path. Anat.* **103**:15, 1886.

"normal" cases were of the active type. Groll and Krampf concluded that the necrotic changes appearing in "germ centers," such as are seen particularly in diphtheria, are brought about by toxins.

Heiberg<sup>43</sup> developed the thesis that phagocytosis is the one important faculty of the "functional centers" of lymphatic tissue, and that these centers have nothing to do with the production of lymphocytes. He based his conclusions on his findings in human tonsils (1923, 1924) and other lymphatic tissues (1928). He considered the secondary nodules as the principal place for the destruction of lymphocytes, and the so-called "germ center cells" he believed were probably reserve phagocytes. The basis for such conclusions was: the parallel increase and decrease of the numbers of mitotic figures and phagocytes filled with debris of lymphocytes; the constant presence of disintegrating lymphocytes in the active type of center, and the absence of disintegrating lymphocytes in the quiescent type of center. Heiberg considered the lymphocyte border of the active type of secondary nodule as a lymphocytic infiltration, such as occurs about a degenerative process.

Wätjen<sup>44</sup> concluded from the results obtained by injecting arsenic intravenously into rabbits, dogs and cats that the secondary nodules were both reaction centers and "germ centers." He found karyorrhexis of the cells of the centers only, and because surrounding cells were not fragmented, he considered that the cell fragments in the center were of "lymphoblastic" origin. He believed that the reaction to arsenic was general, rather than specific for arsenic, and he considered the changes in the centers to be of the same type as those described for diphtheria and those reported by Heineke<sup>45</sup> after experimental use of roentgen rays. In many species of animals exposed to roentgen rays, Heineke had found a rapid appearance of nuclear fragments in the secondary nodules of all the lymphatic tissues. This nuclear fragmentation was followed by the appearance of many mitotic figures and marked phagocytosis. These changes in the secondary nodules were found also by Warthin,<sup>46</sup> Jolly and Rosello<sup>21</sup> and others. Heiberg<sup>47</sup> cited these changes as supporting his contention that the secondary nodules are phagocytic structures primarily.

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43. Heiberg, K. A.: *Virchows Arch. f. path. Anat.* **240**:301, 1923; *Acta otolaryng.* **6**:190, 1924; **7**:3, 1924; *Acta med. Scandinav.* **65**:443, 1927; *Acta path. et microbiol. Scandinav. (supp.)* **5**:43, 1928.

44. Wätjen, J.: *Verhandl. d. deutsch. path. Gesellsch.* **20**:366, 1925; *Virchows Arch. f. Path. Anat.* **256**:86, 1925; **271**:556, 1929.

45. Heineke, H.: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **14**:21, 1905.

46. Warthin, A. S.: *Internat. Clin.* **4**:243, 1906.

47. Heiberg (footnote 43, first reference).

Heilman<sup>48</sup> interpreted his findings in human appendixes in favor of Hellman's hypothesis. Heilman summarized his views<sup>49</sup> by stating that the "secondary follicles" are formed of hyperplastic reticulo-endothelial cells, which are phagocytic for damaged lymphocytes, and act as antibody-forming organs. He considered the "germ center" type of secondary nodule pathologic because it appears after birth.

Parodi,<sup>23</sup> on the basis of a study of 66 spleens of human beings, from 35 cm. fetuses to persons 85 years of age, pointed out that the "lymphoblastic" or "Flemming type" of secondary nodule was rare in such material. He therefore concluded that the lymphoblastic type of secondary nodule was exceedingly sensitive to a state of illness. He further found, as had Downey and Weidenreich<sup>12</sup> and Mollier,<sup>50</sup> that lymphocytes are formed in lymphoid tissue in which no "germ centers" are found. He believed Hellman's hypothesis correct only in that it indicated that the lymph nodule is subject to stimuli from outside the node.

Catania,<sup>51</sup> working in Parodi's laboratory, considered that Hellman's views must be supported on the basis of the appearance of the secondary nodules in Waldeyer's ring and appendix in 33 persons dead of disease.

Rotter<sup>52</sup> examined 200 lymph nodes from 35 human beings and defined 6 types of secondary nodule: (1) the solid, consisting of mature lymphocytes; (2) the epithelioid; (3) the reticular; (4) the lymphoblastic; (5) the necrotic, and (6) the hyaline. He expressed the belief that the secondary nodules arise as a reaction to blood-borne poisons, and that the type of reaction depends on the degree of sensitization of the nodule as brought about by lymphogenous, enterogenous, splenogenous or hematogenous poisons.

Latta<sup>53</sup> concluded, on the basis of human autopsy material, that the light centers of the lymph nodules of the spleen were the result of a degenerative process. He considered the degeneration due to interference with the blood supply of the center of the nodule by the proliferation of the lymphocytes about its border. Latta found that the light centers contained no capillaries, and that they contained more reticular fibers than the surrounding tissue. No mitotic figures were seen in the centers, and little phagocytosis was noted. He had previ-

48. Heilman, P.: *Virchows Arch. f. path. Anat.* **258**:52, 1925.

49. Heilman, P.: *Virchows Arch. f. path. Anat.* **259**:160, 1926.

50. Mollier, S.: *Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. in München.* **29**:14, 1913.

51. Catania, V.: *Haematologica* **8**:221, 1927.

52. Rotter, W.: *Virchows Arch. f. path. Anat.* **265**:596, 1927.

53. Latta, J. S.: *Anat. Rec.* **24**:233, 1923.

ously<sup>54</sup> expressed the belief that the light centers of the lymphatic nodules of the rabbits' intestine were due to interference with the blood supply of the center by proliferation of the cells of the nodule. Such an interpretation of secondary nodules is conceivable on the basis of observations at autopsy in human spleens, in which the commonest types of secondary nodules are the reticular, epithelioid and hyaline. It is not applicable to the "germ center" type of secondary nodule, which contains abundant capillaries, mitotic figures throughout, abundant active phagocytes and fewer reticular fibers than the surrounding tissue.

Uchino,<sup>55</sup> working in Aschoff's laboratory, experimented extensively with subcutaneous and intramuscular injections of nutrose, mixtures of nutrose and bacteria and bacteria alone. He found fairly uniform changes in the lymphatic nodules of the lymph nodes and spleen after repeated injections. Their centers were increased in size and contained more fragmented nuclei and more mitotic figures. The degree of change was proportional to the number of injections. The medullary cords and the internodular cortical tissue took no appreciable part in the reaction. Uchino offered his results in support of Wätjen's<sup>56</sup> contention that both Flemming's and Hellman's views were in part correct. Aschoff<sup>57</sup> expressed a similar view.

Maximow's extensive observations of connective tissue<sup>58</sup> and tissue cultures,<sup>59</sup> led him to support Flemming's theory. Maximow<sup>60</sup> summarized his ideas of the secondary nodules of lymph nodes in Cowdry's *Special Cytology*. He reported there the results of a cytologic study of the "germ centers" of the lymph nodes of the cat. He described various-sized secondary nodules with varying cellular content. He interpreted these variations as cyclic changes from a resting stage to one of active production of lymphocytes and macrophages. The active phase was followed by stages of regression to the resting stage. In discussing the functional significance of the "germ centers," Maximow stated, "It is quite probable that the lymphoid tissue in general and the germ centers in particular may take part in the defense reactions of the organism. It is also certain that the germ centers are not abso-

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54. Latta, J. S.: *Am. J. Anat.* **24**:159, 1921.

55. Uchino, S.: *Beitr. z. path. Anat. u. z. allg. Path.* **74**:405, 1925.

56. Wätjen (footnote 44, first reference).

57. Aschoff, L.: *Lectures on Pathology: Reticulo-Endothelial System*, New York, Paul B. Hoeber, 1924, p. 1.

58. Maximow, A.: *Development of the Blood*, in von Möllendorff, Wilhelm: *Handbuch der Gewebelehre der Menschen*, Berlin, Julius Springer, 1929, vol. 2, pt. 1, p. 232.

59. Maximow, A.: *Arch. f. mikr. Anat.* **97**:283 and 314, 1923.

60. Maximow, A.: *Lymphocyte and Plasma Cell*, in Cowdry, E. V.: *Special Cytology*, New York, Paul B. Hoeber, 1928, vol. 1, p. 321.

lutely necessary for the regeneration of lymphocytes, as in the embryo and in the new-born infant, they are not clearly developed." He concluded his discussion with the following quite inconsistent statement: "In view of the described regular cyclic growth phenomena in the germ centers their importance as the *principal* places of regeneration of lymphocytes, as formulated by Flemming, has to be maintained."

That the inconsistency may be due to a misconception regarding the frequency of the occurrence of the secondary nodules of the Flemming type is indicated by a statement made on page 332: "They (germ centers) may disappear and arise again according to the changing *physiological* conditions. . . . As a rule all germ centers of a lymph node, perhaps of the whole body, show similar conditions at a given time." It is well known that secondary nodules of the active type are common in laboratory animals. An explanation is seen in the work of Manfredi<sup>61</sup> and in that of Uchino.<sup>54</sup> Manfredi cultured bacteria from lymph nodes of 88.6 per cent of 88 "normal" animals. Uchino produced proliferative changes in lymphatic nodules by subcutaneous injection of bacteria. These observations, coupled with those concerning the scarcity of secondary nodules of the active type at times of life when man is least exposed to infections, would indicate that their presence is due to pathologic stimuli. There is abundant evidence in the literature (Uchino,<sup>55</sup> Catania,<sup>51</sup> Ehrich,<sup>62</sup> Parodi<sup>28</sup>), and my own observations indicate, that the conditions of the lymph nodules may vary according to the location in the body and even the location in the node.

Lang<sup>63</sup> investigated lymph nodes of rabbits in which myeloid metaplasia had been produced experimentally. He concluded that the secondary nodules of lymphatic tissue contained 2 undifferentiated mesenchymal types of cell, the lymphoblast and the reticular cell, each differentiating according to its stimulus. He considered both Flemming's and Hellman's theories correct in part.

Ehrich reviewed the anatomy<sup>31</sup> and embryology<sup>21</sup> of human lymph nodes and described large nodules in the parenchyma of the lymph nodes of rabbits and human beings, which he designated pseudo-secondary nodules. He described these pseudosecondary nodules as a transitional stage between secondary nodules of the Flemming type and "lymphoid tissue" (Aschoff) and between secondary nodules of the solid type and "lymphoid tissue." He considered the pseudosecondary nodule to be the chief site of lymphocyte production in the embryo, in which they first appear about the twenty-second week, at the time the solid secondary nodules appear.

61. Manfredi, L.: Virchows Arch. f. path. Anat. **155**:335, 1899.

62. Ehrich, W.: J. Exper. Med. **49**:361, 1929.

63. Lang, F. J.: Folia haemat. **36**:31, 1928.

Ehrich,<sup>64</sup> using rabbits, produced, by subcutaneous injection of small amounts of cultures of a staphylococcus of very low virulence, a hyperplasia of a local lymph node and a lymphocytosis. The lymphocytosis appeared coincidentally with the regression of the secondary nodules of the Flemming type in the regional lymph node. The secondary nodules of the Flemming type became prominent and were increased in number later, after the lymphocytosis had disappeared. This increase in the number of the secondary nodules appeared to be associated with abscess formation at the site of injection. After injecting killed cultures of staphylococci intravenously into rabbits, Ehrich<sup>62</sup> found a peak in the leukocytosis and lymphocytosis on the tenth day paralleled by an increase in the weight of the peripheral lymph nodes and in that of the spleen. He found a great increase in the number of Flemming's nodules in the spleen and some increase in the size of the nodules. The increase in the number of Flemming's nodules in the peripheral lymph nodes was not so marked. Ehrich's conclusions<sup>65</sup> were that Flemming's theory could not be supported in its original form and questionably in a modified form.

Schillaci<sup>66</sup> studied the behavior of lymph nodes of guinea-pigs in acute and prolonged inanition. He found that the lymphocyte mantles of the secondary nodules decreased as starvation was prolonged, and that, when the animals were fed again, these lymphocyte mantles returned coincidentally with an increase in the number of mitotic figures in the secondary nodule. He concluded that these occurrences could be explained best by Flemming's theory that the centers have a lymphocyte-forming function.

In apparent contradiction to the foregoing interpretation are the observations of Gottesman and Gottesman.<sup>67</sup> They studied autoplasmic lymph node transplants in albino rats and noted the parallel development of reticulum and lymphocytes with transitional stages between. The lymphatic nodules were formed late in the regeneration, and only in the latest stages studied (eighth day) was there beginning formation of "germinal centers."

#### MATERIALS AND METHODS

General studies of the structure of the secondary nodules of lymphatic tissue were made on approximately 1,500 lymph nodes removed from 500 cadavers within four hours after death. The ages of the deceased varied from 6 months in utero to 88 years. Nodes were removed from all the regions in many cases; in others, only nodes from a visceral and a peripheral group were taken. Approximately

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64. Ehrich, W.: *J. Exper. Med.* **49**:347, 1929.

65. Ehrich, W.: *Beitr. z. path. Anat. u. z. allg. Path.* **86**:285, 1931.

66. Schillaci, G.: *Haematologica* **9**:397, 1928.

67. Gottesman, J. M., and Gottesman, J.: *Proc. Soc. Exper. Biol. & Med.* **25**:484, 1928.

200 of the nodes were removed from the pyloric region of the stomach. Spleens in all the cases and submucous lymphatic tissue in many of the cases were studied. The lymph nodes and spleens of 30 rabbits and of several cats, dogs, guinea-pigs and sheep were examined. The human material, as a routine, was fixed in modified Orth's solution, embedded in paraffin and stained with hematoxylin and eosin. The animal material was fixed in 4 per cent formaldehyde. For cytologic studies, Hitchcock and Ehrich's<sup>68</sup> modification of the Pappenheim stain was used on material fixed in Zenker's solution. The Perdrau<sup>69</sup> silver method was used for reticulum.

In addition to the aforementioned materials, lymph nodes, tonsils and appendixes removed at operation were studied in fresh, frozen sections stained with Terry's polychrome methylene blue and in formaldehyde-fixed, frozen sections stained with hematoxylin and eosin.

A special study was made of the lymph nodes resected with a part of the stomach for gastric carcinoma or for gastric ulcer. There were 116 specimens from partial gastrectomies; 98 of these were from gastrectomies for gastric carcinoma and 18 from those for gastric ulcer. The lymph nodes from 43 of the cases of carcinoma had previously been sectioned for a study of metastasis by MacCarthy and Blackford.<sup>70</sup> My studies of the lymph nodes in these 43 cases were made on the fixed, frozen sections stained with hematoxylin and eosin that had been prepared in 1912. The remainder of the nodes from the 116 specimens were taken from specimens that had been preserved in formaldehyde for periods varying from two weeks to seven years. Many nodes were sectioned serially. Sections of the gastric lesion were studied in all cases. The total number of nodes studied from the 116 specimens was 593. Only those nodes not completely replaced by carcinoma were used. In the entire group of persons from whom the specimens came, there were 108 men and 8 women; the average age was 56, the ages ranging from 26 to 76 years.

#### RECORDED OBSERVATIONS ON RELATIONSHIP BETWEEN GASTRIC LESIONS AND HYPERPLASIA OF REGIONAL LYMPH NODES

Investigations of the relationship between gastric lesions and regional lymph nodes have been concerned chiefly with metastatic involvement of the nodes. Cunèo,<sup>71</sup> however, noted that "hyperplastic glands" were sometimes present in the region of gastric carcinoma without carcinomatous involvement of the nodes. Carle and Fantino<sup>72</sup> had made the same observation. Lengemann<sup>73</sup> mentioned large nodes in the region of a benign ulcer of the stomach and large noncarcinomatous nodes in the region of a gastric carcinoma. MacCarty and Blackford<sup>70</sup> made similar observations. None of these workers commented on secondary nodules, and I have been able to find in the

68. Hitchcock, C. H., and Ehrich, W.: *Arch. Path.* **9**:625, 1930.

69. Perdrau, J. R.: *J. Path. & Bact.* **24**:117, 1921.

70. MacCarty, W. C., and Blackford, J. N.: *Ann. Surg.* **55**:811, 1912.

71. Cunèo, B.: *De l'en vahissement du système lymphatique dans le cancer de l'estomac*, Thèse de Paris, 1900.

72. Carle, A., and Fantino, G.: *Arch. f. klin. Chir.* **56**:217, 1898.

73. Lengemann: *Arch. f. klin. Chir.* **68**:382, 1902.



literature no report concerning the frequency of the occurrence of secondary nodules of the active type in the lymph nodes in gastric lesions.

#### AUTHOR'S OBSERVATIONS

In the general study of postmortem lymphatic tissue, the outstanding feature was the extreme variability of the structures that might be considered "normal." The variation in lymph nodules was most striking. Secondary nodules of the active type, that is, the type described by Fleming, were exceedingly rare in persons over 30 years of age in the absence of long-standing, mild, chronic inflammatory processes.

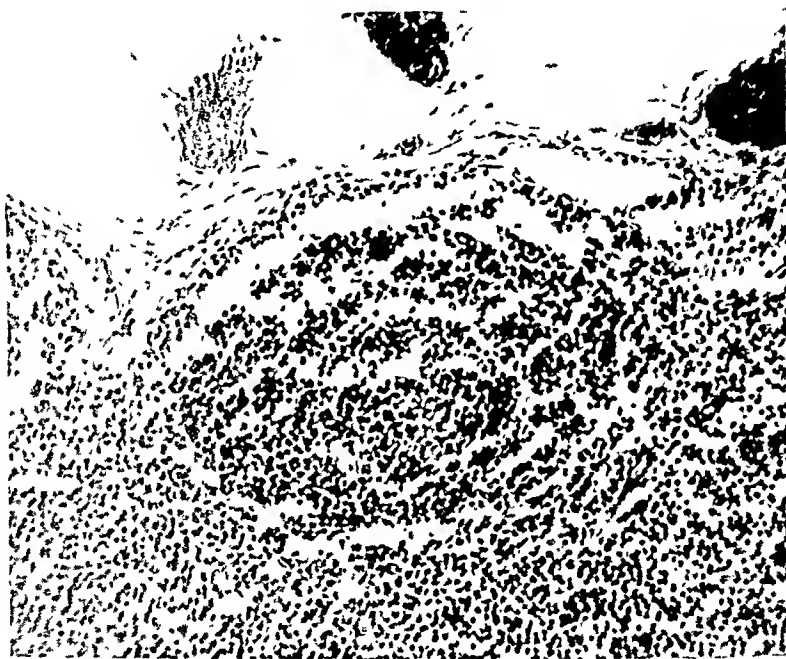


Fig. 1.—A primary lymphatic nodule without a secondary nodule;  $\times$  225.

Lymph nodules without secondary nodules were frequently seen in lymph nodes. The necrotic type of secondary nodule was occasionally found, but by far the greater number of secondary nodules were of the hyaline, reticular and epithelioid types. All of the secondary nodules seen could be designated according to the classification for those of the spleen by Groll and Krampf<sup>41</sup> and for those of lymph nodes by Rotter.<sup>52</sup> The various types of secondary nodules are pictured in figures 1, 2, 3, 4, 5, 6 and 7. The rarity of the active type and the abundance of the inactive type of secondary nodule in the age group beyond 30 years of age agree with the findings of Groll and Krampf<sup>41</sup> and Parodi<sup>23</sup> for the spleen, and with those of Meek<sup>74</sup> for lymph nodes.

74. Meek, W. O.: *Quart. J. Med.* 3:395, 1910.

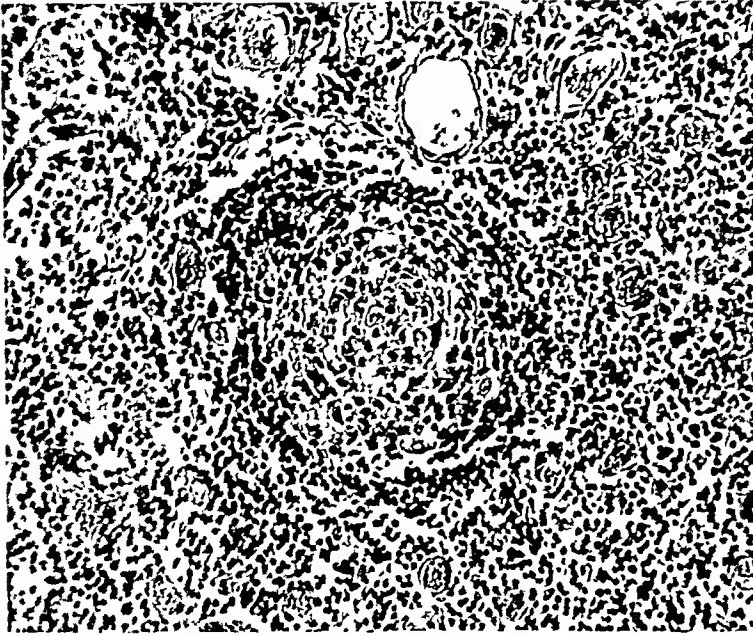


Fig. 2.—A lymphatic nodule with a secondary nodule of the active type;  $\times 200$ .

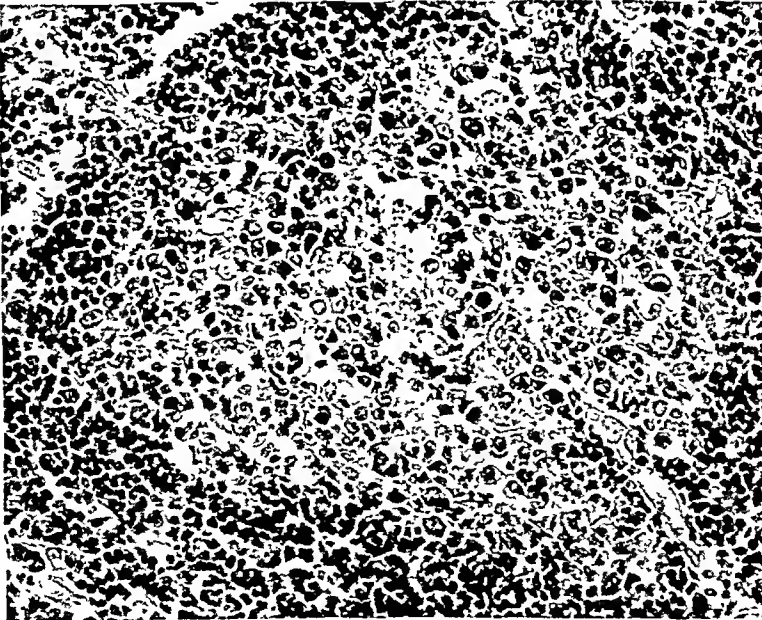


Fig. 3.—The active type of secondary nodule;  $\times 300$ .

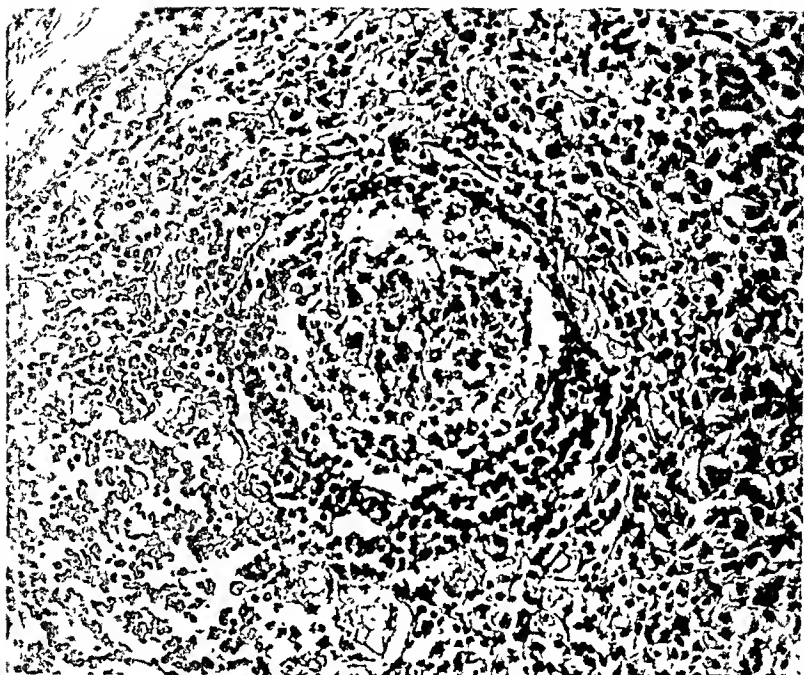


Fig. 4.—A lymphatic nodule with a secondary nodule of the reticular type. The tissue about the nodule is carcinoma;  $\times 250$ .



Fig. 5.—A lymphatic nodule with a secondary nodule of the epithelioid type;  $\times 215$ .



Fig. 6.—A lymphatic nodule with a secondary nodule of the hyaline type;  $\times 215$ .



Fig. 7.—A lymphatic nodule with a secondary nodule of the necrotic type;  $\times 275$ .

The cases for special study were divided into 3 groups as indicated in table 1. Group 1 consisted of 55 cases of carcinoma of the stomach in which 269 lymph nodes were obtained. This group was studied particularly for the case incidence of the active type of secondary nodule. The number of nodes in each case varied from 1 to 12; the average number was 5. In all but one case of this group there were found well formed secondary nodules of the active type. In many of the cases, all the nodes secured contained these secondary nodules. In the cases from which many nodes were secured, there was usually one or more nodes that did not contain the active type of secondary nodule, but did contain primary nodules without secondary nodules, or secondary nodules of the reticular, hyaline or epithelioid type. The case in which no secondary nodules of the active type were found yielded 6 nodes containing the reticular type of secondary nodule. No explanation for this peculiarity could be found.

Group 2 consisted of 43 cases of gastric carcinoma and was selected for an analysis of the incidence of secondary nodules of the active type in each case. This group was suitable for such an analysis because all the lymph nodes resected were available for study. The number of nodes not obliterated by carcinoma in each case varied from 1 to 17; the average number was 6.3. All but 1 of the 43 cases yielded nodes containing secondary nodules of the active type. This case yielded but a single node for study. Of the total of 270 nodes obtained, 138 contained large secondary nodules of the active type. Eighty-two of the nodes contained smaller, but none the less typical, secondary nodules of this type. In 50 of the nodes no secondary nodules of the active type were found.

Group 3 consisted of 18 cases of gastric ulcer, in which 54 nodes were obtained from the resected portions of the stomachs. This group was studied as a control on the cases of carcinoma. All of the cases in this group yielded nodes containing large, well formed secondary nodules of the active type. In most of the cases, all the nodes examined contained this type of secondary nodule. The only difference between this group and that of cases of carcinoma was the smaller number of nodes obtained. This might be expected because of the smaller lesions in ulcer. The number of nodes in each case varied from 1 to 8; the average number was 3.

The histologic and cytologic changes were essentially the same in the three groups, and they will be described together. Changes in the gastric wall were strikingly uniform qualitatively. Sections from the border and the base of all the carcinomas and ulcers showed varying degrees of infiltration with plasma cells and eosinophils. The plasma cell infiltration was most marked in the mucosa and submucosa about:

TABLE 1.—*Relationship of Secondary Nodules of Active Type in Regional Lymph Nodes to Gastric Lesions*

Group	Number of Cases	Average Age of Patients, Years	Number of Nodes	Number of Cases with Secondary Nodules of Active Type
1. Carcinoma.....	55	57.6	269	54
2. Carcinoma.....	43	54	270	42
3. Ulcer.....	18	57	54	18
Totals.....	116		593	114
Average age.....		56		

TABLE 2.—*Incidence of Nodes Containing Active Secondary Nodules in Group 1, Cases of Gastric Carcinoma in Which Stomachs Were Resected*

Case	Age of Patient, Years	Sex	Number of Nodes	Number of Nodes Containing Secondary Nodules of Active Type	Number of Nodes Without Secondary Nodules of Active Type
1	52	F	5	5	0
2	54	F	6	5	1
3	54	M	7	5	2
4	72	M	3	3	0
5	55	M	12	8	4
6	75	M	2	2	0
8	65	M	6	5	1
9	64	M	3	1	2
10	76	M	1	1	0
11	50	M	7	5	2
12	58	M	10	7	3
13	65	M	4	4	0
14	58	M	1	1	0
15	54	M	1	1	0
16	66	M	2	2	0
17	69	M	1	1	0
18	60	M	11	10	1
19	54	M	4	4	0
20	52	M	6	5	1
21	55	M	3	3	0
22	57	F	4	4	0
23	55	M	4	4	0
24	40	M	9	7	2
25	71	M	2	2	0
26	44	M	2	2	0
27	56	M	2	2	0
28	36	M	7	6	1
29	64	M	9	6	3
30	57	M	2	2	0
31	57	M	1	1	0
33	54	M	10	6	4
34	56	F	3	3	0
35	55	M	8	4	4
36	57	M	1	1	0
38	62	M	1	1	0
39	63	M	1	1	0
40	53	M	1	1	0
41	60	M	10	5	5
42	49	M	6	6	0
43	63	M	6	5	1
44	50	M	10	8	2
45	43	M	4	4	0
46	57	M	2	2	0
47	59	M	3	3	0
48	63	M	9	8	1
49	62	M	1	1	0
50	57	M	12	10	2
51	63	M	10	2	8
52	64	F	4	4	0
53	62	M	9	9	0
55	42	F	8	5	3
56	64	M	5	5	0
57	53	M	6	0	6
58	61	M	1	1	0
59	67	F	1	1	0

TABLE 3.—*Incidence of Nodes Containing Active Secondary Nodules in Group 2, Cases of Gastric Carcinoma in Which Stomachs Were Resected*

Case*	Number of Nodes Containing Lymphoid Tissue	Number of Nodes Containing Secondary Nodules		Number of Nodes Containing No Secondary Nodules
		Large	Small	
1	17	5	6	6
2	9	3	4	2
3	9	8	0	1
4	4	3	1	0
5	3	2	1	0
6	7	0	2	5
7	1	1	0	0
8	4	4	0	0
9	6	5	0	1
10	2	2	0	0
11	10	6	4	0
12	1	1	0	0
13	1	0	0	1
14	4	1	0	3
15	10	3	5	2
16	5	4	1	0
17	6	1	3	2
18	7	7	0	0
19	10	9	1	0
20	6	4	2	0
21	4	2	0	2
22	9	7	2	0
23	7	2	2	3
24	6	4	2	0
25	3	2	0	1
26	6	2	3	1
27	8	2	3	3
28	3	1	1	1
29	8	2	4	2
30	1	1	0	0
31	6	0	5	1
32	4	3	1	0
33	4	3	1	0
34	11	5	3	3
35	8	3	4	1
36	8	2	4	2
37	11	1	9	1
38	10	7	1	2
39	7	4	1	2
40	7	5	1	1
41	3	2	1	0
42	4	0	2	2
43	10	7	3	0
	270	136	83	51

\* All 43 cases had nodes containing secondary nodules of the active type.

the borders of the ulcer, whether it was benign or malignant. In many of the cases there were accumulations of plasma cells throughout the muscularis. The eosinophils were present in large numbers throughout the immediate region of the ulcerated portion of the lesion. There were few polymorphonuclear neutrophilic granulocytes in most of the lesions. Three of the carcinomas were of the nonulcerated polypoid type; sections of two of these were marked by a greater number of polymorphonuclear neutrophils than the ulcerating lesions. Plasma cells and eosinophilic granulocytes were also present in these sections.

TABLE 4.—*Incidence of Nodes Containing Active Secondary Nodules in Group 3, Cases of Gastric Ulcer in Which Stomachs Were Resected*

Case	Age of Patient, Years	Sex	Number of Nodes	Number of Nodes Containing Secondary Nodules of Active Type	Number of Nodes Without Secondary Nodules of Active Type
1	38	M	2	2	0
3	67	M	6	6	0
4	66	M	4	4	0
5	65	M	1	1	0
6	46	F	8	5	3
8	44	M	2	2	0
9	56	M	1	1	0
10	64	M	3	3	0
11	61	M	2	2	0
12	53	M	6	6	0
13	66	M	6	3	3
14	65	M	1	1	0
16	57	M	1	1	0
18	67	M	2	2	0
19	61	M	3	3	0
20	26	F	1	1	0
22	58	M	4	4	0
23	65	M	1	0	1
18 cases.....			54	47	7

No definite quantitative correlation between the changes in the gastric wall and the changes in the nodes could be established. But such a relationship was suggested by case 9 in group 1, which was that of a polypoid carcinoma with little chronic inflammatory reaction, case 6 in group 2 and case 18 in group 1, which showed little chronic inflammatory reaction. The nodes from these cases contained few small secondary nodules of the active type; a larger proportion of the nodes than in other cases contained no secondary nodules. Case 58 in group 1 similarly suggests a quantitative relationship. The nodes in this case were numerous and contained unusually large and numerous secondary nodules of the active type (fig. 9). The gastric mucosa and submucosa in this case contained unusually large numbers of plasma cells (fig. 10).



The changes in the lymph nodes containing secondary nodules of the active type were also qualitatively uniform. In group 2, 81.5 per cent of the nodes not completely obliterated by carcinoma contained secondary nodules of the active type. These nodes were characterized also by the presence of many plasma cells in the capsules, sinuses and lymphatic cords. All of the secondary nodules of the active type in all cases were marked by the presence of mitotic figures and active phagocytes. The size of the secondary nodules was directly proportional to the number of mitotic figures and active phagocytes within the secondary nodules. Acute inflammatory reaction, as evidenced by the presence of neutrophilic polymorphonuclear granulocytes, was rarely found. In the presence of more than a few polymorphonuclear leukocytes in the node, secondary nodules of the active type were not found. A post-



Fig. 8.—A lymph node from a stomach resected for gastric carcinoma in a man, aged 58. This is the typical node found in both carcinoma and ulcer;  $\times 11.5$ .

mortem study of the nodes remaining in the region of the stomach after partial gastrectomy indicated that acute inflammatory processes cause a regression of secondary nodules of the active type. Such studies were made in 14 cases (nos. 5, 9, 10, 11, 13, 20, 29 and 31 in group 1 and nos. 1, 3, 4, 5, 9 and 10 in group 3). In but 2 of these cases (nos. 1 and 9, group 3) were gastric nodes secured post mortem that contained well preserved secondary nodules of the active type. Sections of the stomach from case 9, group 3, contained no evidence of acute inflammation. The patient in case 1, group 3, died of hemorrhage shortly after operation. Nodes in all the other cases of this group contained secondary nodules of various types other than the active. The most common type was the solid nodule. The next most frequent type was the epithelioid, then the hyaline. No necrotic types were found. The interpretation of these findings might be either that

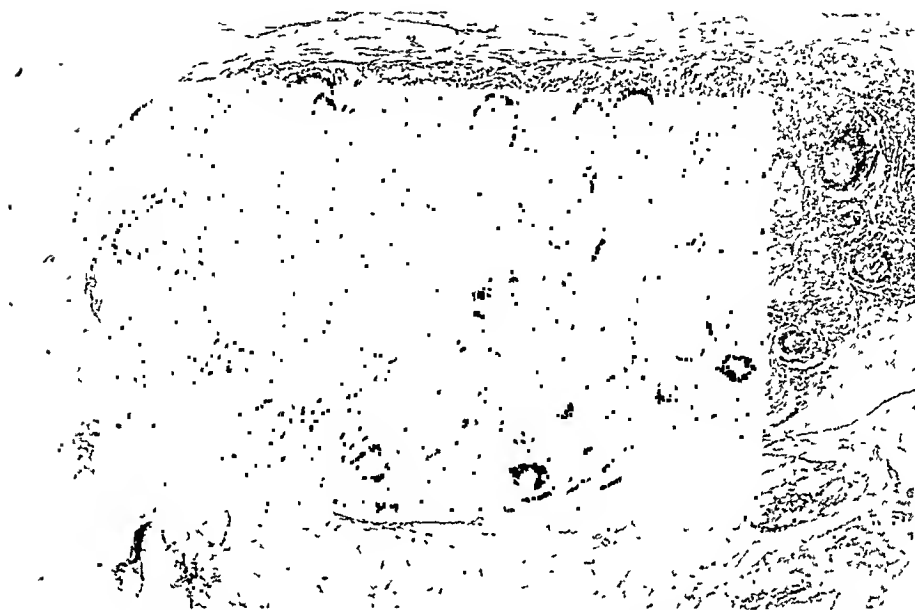


Fig. 9.—A lymph node from a stomach resected for gastric carcinoma in a man, aged 61. The secondary nodules of the active type are extremely large and numerous;  $\times 11.5$ .

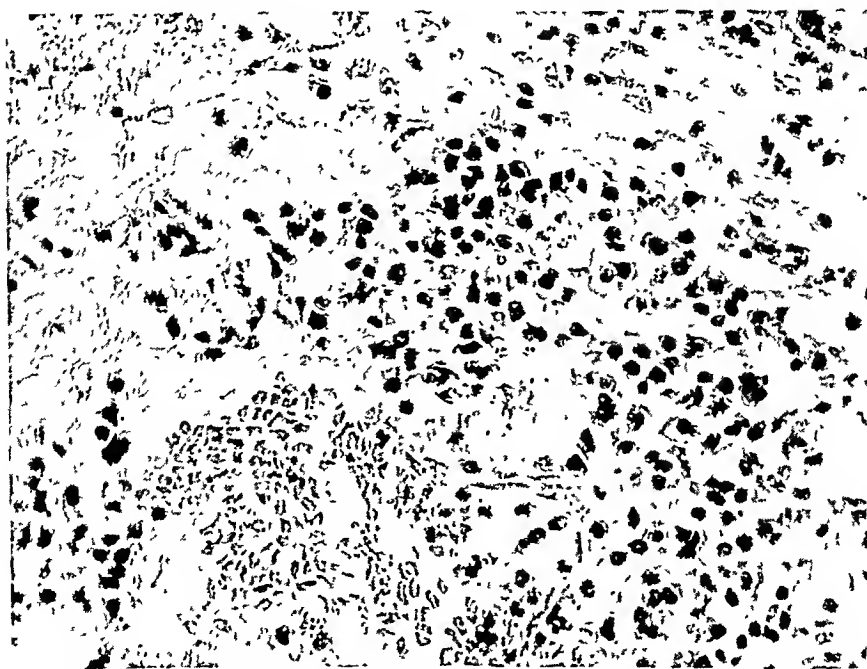


Fig. 10.—A section from the gastric wall in the same case as the lymph node shown in figure 9. Note the plasma cell infiltration;  $\times 400$ .

the nodes secured post mortem did not drain the original lesion, or that the acute inflammatory reaction resulting from the operation caused a regression of the secondary nodules. No correlation could be established between the degree of fibrosis or of lymphocytic infiltration of the lesion and the reaction in the lymph nodes.

#### SUMMARY

An examination of 1,500 lymph nodes from persons dying of various causes revealed few secondary nodules of the active type in the age group beyond 30 years. Examination of 592 lymph nodes from the portions of 116 stomachs resected from carcinoma and benign ulcer revealed that 114 (98.2 per cent) of the specimens contained lymph nodes with secondary nodules of the active type. The average age of the persons in this group was 56 years. In a group of 43 cases, 81.5 per cent of the nodes not completely obliterated by carcinoma contained active secondary nodules. Microscopically, the gastric lesions were characterized by an infiltration with plasma cells and eosinophilic leukocytes. The lymph nodes were characterized by the presence of plasma cells in increased numbers and by the presence of well formed secondary nodules containing many mitotic figures and active phagocytes.

#### COMMENT

Flemming's observations were made on a small amount of material that permitted him to draw but one conclusion. He observed apparently but one type of secondary nodule, that is, the type that he called physiologically a "germinal center." Some subsequent observers failed to substantiate Flemming's findings because they used limited material of a different nature. Since there has been abundant demonstration that there exist several types of secondary nodule, different in structure and different in significance, the designation of all these types of secondary nodule by a single term implying a single significance is no longer justified. If the material used by various workers is considered, many of the apparent conflicts in the literature disappear. There remain, however, several objections to the acceptance of Flemming's theory.

The contention that the secondary nodules of lymphatic tissue are the chief places for the formation of lymphocytes must be denied on the basis of the following facts: 1. Lymphocytes are formed abundantly in the absence of secondary nodules. 2. Lymphocytes are invariably present before secondary nodules are formed. 3. Lymphocytes are formed elsewhere than in secondary nodules at times of life when secondary nodules may be present.

The contention that the secondary nodules of lymphatic tissue are physiologically places for the formation of lymphocytes must be questioned on the basis of the strong presumptive evidence in the literature that the secondary nodules of the active type arise at a time when, and in locations where, pathologic stimuli cannot be eliminated as a factor. Furthermore, there is evidence that the secondary nodules of the active type can be increased in number and size by pathologic stimuli.

The contention that secondary nodules of the active type give rise to lymphocytes must be admitted on the basis of the careful cytologic studies of the hematologists.

#### CONCLUSIONS

Secondary nodules of the active type ("germ centers," the Flemming type of secondary nodule) are rarely found in lymph nodes of man beyond 30 years of age in the absence of long-standing, mild, chronic inflammatory processes.

Secondary nodules of the active type are common (98.2 per cent of cases) in lymph nodes associated with chronic ulcerating lesions of the stomach in man beyond 30 years of age.

This circumstantial evidence and the histologic picture indicate an etiologic relationship between the pathologic process in the gastric wall and the production of secondary nodules of the active type in the near-by lymph nodes.

This evidence is offered in support of the contention that the secondary nodules of lymph nodes arise as the result of pathologic processes.

These observations on human material support the conclusion drawn from experimentation on animals, that the secondary nodules of lymphatic tissue are reaction centers, and should increase the skepticism with which Flemming's hypothesis is viewed.

# EXPERIMENTALLY PRODUCED FOCAL (DENTAL) INFECTION IN RELATION TO CARDIAC STRUCTURE \*

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To our knowledge there has been no report in the literature of an experimentally controlled attempt to correlate the effect of focal infection with cardiac hypertrophy or to determine myocardial response to toxic injury and infection under stress and strain. Rosenow and Meisser<sup>1</sup> reported the experimental production of renal stones in dogs following the establishment of a permanent focal infection in the canine teeth of the animals with a micro-organism which they believed to possess a selective affinity for renal tissue. The method appealed to us as a possible means of studying experimentally the relation, if any, that may exist between chronic focal infection and disease of the heart.

In this paper, we discuss the following problems: (1) the relationship, if any, between experimentally produced focal (dental) infection and cardiac hypertrophy in dogs; (2) the relative effects of exercise on the hearts of such infected and noninfected dogs; (3) the reliability of the various means of expressing cardiac hypertrophy, and (4) other pathologic changes, cardiac and extracardiac, found in the experimental animals.

The literature on the cause of cardiac hypertrophy is voluminous, and no attempt to review it is made here. A few of the principles and opinions concerned are alone noted. Külbs,<sup>2</sup> in reviewing the literature, found a general acceptance of the statement that a work hypertrophy exists, and concluded that an increase in the relative and in the absolute weight of the heart occurs with exercise, without a corresponding increase in the weight of the body muscle. Horvath<sup>3</sup> denied that hypertrophy is due to increased work. Albrecht<sup>4</sup> found no constant

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\* From the Department of Medicine, University of Oregon Medical School.

1. Rosenow, E. C., and Meisser, J. G.: *Arch. Int. Med.* **31**:807, 1923.

2. Külbs: *Arch. f. exper. Path. u. Pharmakol.* **55**:288, 1906.

3. Horvath, A.: *Ueber die Hypertrophie des Herzens*, Leipzig, W. Braunnüller, 1897; quoted by Stewart (footnote 5).

4. Albrecht, E.: *Der Herzmuskel und seine Bedeutung für Physiologie, Pathologie und Klinik des Herzens*, Berlin, Julius Springer, 1903; quoted by Stewart (footnote 5).

relation between function and hypertrophy. There is, however, he said, an increase in the connective tissue stroma, and the vacuolated muscle cells show degeneration of the anisotropic portions and increase in the isotropic portions of the muscle elements, with, often, a doubling of the nuclei. Albrecht considered such changes a response to infection. Stewart<sup>5</sup> stated that hypertrophy induced by production of aortic insufficiency involves all chambers of the heart. The hypertrophy is greatest absolutely in the left ventricle, and is next greatest, in order, in the septum, right ventricle and auricles. The greatest relative increase is also seen in the left ventricle, but the auricle shows a greater relative increase than either the septum or the right ventricle. Herrmann<sup>6</sup> concluded that the left ventricle has the greatest relative and the greatest absolute hypertrophy in experimental aortic insufficiency. He stated, further, that older dogs are often refractory to hypertrophy, and that young dogs show more uniformity of response; that the presence or the absence of intracardial infection is an important factor, and that the solution of the problem of cardiac hypertrophy in general is still out of reach because, presumably, a number of factors are active in the process. Goldberger<sup>7</sup> found that the muscle cell of the hypertrophied heart is larger than normal, and stated that the increase is a volume increase. Tangl,<sup>8</sup> after using a maceration process on specimens from the left ventricular wall, concluded that in physiologic growth as well as in pathologic hypertrophy the greater the absolute weight of the heart the greater is the cross diameter of the cells.

#### EXPERIMENTAL TECHNIC

The dogs used in these experiments constituted an unselected group, except that the age of each approached 9 months, in order that the canine teeth might be sufficiently developed to permit of carrying on this work and the hearts not refractory to infection, and also so that they, in general, might represent an average group comparable to the normal canine standards determined by Herrmann.<sup>9</sup> Thirty-one dogs, divided into three groups, were used.

The twelve dogs of the inoculated group were subjected to a rigidly followed operative technic by Dr. Frank Mihnos, the attending oral surgeon of the Multnomah County Hospital, which may be summarized as follows:

5. Stewart, H. A.: *J. Exper. Med.* **13**:187, 1911.

6. Herrmann, G. R.: *Am. Heart J.* **1**:485, 1926.

7. Goldberger, B.: *Virchows Arch. f. path. Anat.* **103**:88, 1886; quoted by Tangl (footnote 8).

8. Tangl, Franz: *Virchows Arch. f. path. Anat.* **116**:432, 1889.

9. Herrmann (footnote 6, p. 213).

The dog was anesthetized with ether; the lower canine tooth, or teeth, was isolated with rubber dam and the parts swabbed with iodine. The distal half of the tooth was removed with dental saw and rongeur forceps. The pulp was then removed, the bleeding stopped, and the cavity dried. The cavity was then filled with a streptococcus culture and a pulp point put in place and covered with a small pledget of sterile cotton; a dental cement covering was then placed over the cotton and the tooth permanently filled with silver amalgam.

The streptococcus culture used was isolated by Dr. Benson, of the department of pathology, from antral tissues removed from a patient suffering with chronic hyperplastic sinusitis. It had been previously used by him in an experimental attempt to produce arteriosclerosis in monkeys. The organism was identified by cultural and microscopic characteristics as a nonhemolytic, green-producing, gram-positive type of streptococcus, corresponding in its sugar fermentations to *Streptococcus mitis*. We chose it because, in Dr. Benson's experiments, the hearts of his inoculated animals showed patchy degenerative changes of the myocardium, and we hoped that it might have a selective action on the heart muscle of our dogs. The injected material was a twenty-four hour culture of this organism, washed in physiologic solution of sodium chloride.

The twelve dogs of the control group were selected as the others had been and were subjected to the same care and treatment with the exception of not being inoculated.

The normal dogs, seven in number, were likewise of the same age. They were secured after death and were weighed, and the fresh hearts, weighed, were kept for further study. This series was started with the idea of continuing the inclusions to an indefinite number. When it was found, however, that the series corresponded, practically without exception, to the large group of two hundred normal dogs reported on by Herrmann,<sup>9</sup> it was discontinued.

The inoculated and the control dogs were subjected to stress and strain by daily exercise to exhaustion. To provide such exercise, an electric treadmill, having an inclined canvas belt and two screened cages, was constructed. Each dog was exercised for a period of fifteen minutes daily, six days per week. The tread inclined at an angle of 20 degrees to the horizontal and moved at a rate of 5.4 miles per hour. The average energy thus expended by each dog in his daily run was 22,800 foot-pounds, and reached the sum total of approximately 3,000,000 foot-pounds during the entire experimental period, which averaged 1,550 minutes of exercise per dog. The daily exercise was sufficient to fatigue each dog markedly. The amount of exercise needed to bring about fatigue varied, for especially did the inoculated dogs show exhaustion more quickly than the control dogs.

The dogs were divided into five groups and placed in pens, each provided with an ample outdoor runway and a steam-heated room, maintained at approximately 65 F. The ration provided was a balanced commercial food product (Kibbled Cakes). Hamburger steak was fed at frequent intervals. Water was constantly provided in the pens. Carbon tetrachloride was used as indicated for tapeworm infestation on diagnosis. The dogs were washed at intervals of a fortnight. Every effort was put forth to keep the dogs in the best possible physical condition during the entire course of the experiment. The animals were weighed before running, at intervals during the course of the experiment, and at death.

#### METHODS OF EXAMINATION

Roentgenograms of the teeth of the inoculated and of the control series of dogs, made at intervals, informed us of the presence and progress of the dental infections. Postmortem examinations of the jaws for infected areas were made.

Animals dying during the course of the experimentation were subjected to the same postmortem treatment as animals killed by asphyxia during deep ether anesthesia at the conclusion of the experimental period. An immediate necropsy of the thoracic and abdominal viscera was made, and the presence or the absence of gross pathologic changes noted. The upper and lower jaws were saved for dissection and further investigation. The heart was removed from the pericardial sac and prepared according to the method of Lewis.<sup>10</sup> This method may be summarized as follows:

1. The parietal pericardium was removed.
2. The vessels were cut short.
3. The cavities were washed free of clots and drained.
4. The heart was weighed.
5. The vessels were ligated.
6. The cavities were distended with dilute solution of formaldehyde U. S. P. (1:10) injected through the cardiac wall with a hypodermic needle.
7. The heart was immersed in dilute solution of formaldehyde (1:10) for from five to seven days, depending on the size.
8. The orifices were opened, and the heart was drained and washed in running water for from one to two days.
9. The heart was placed in 70 per cent alcohol until its weight reached normal or until a maximum weight that was still less than normal was reached.
10. The epicardium, subepicardial fat, coronary vessels, valves and chordae tendineae were removed.
11. The auricles and ventricles were separated.
12. The cleaned ventricles were separated into left ventricular, right ventricular and septal portions by a series of cuts parallel to and tangential to the septum.
13. The auricles were cleaned.
14. The heart portions were placed in water and drained.
15. The heart portions were weighed individually.

*Estimation of Cardiac Hypertrophy.*—We investigated four commonly used methods of estimating cardiac hypertrophy in order to adjudge the relative cardiac effects of our experiments on inoculated

10. Lewis, Thomas: Heart 5:367, 1913-1914.



and noninoculated dogs. These methods of expressing cardiac hypertrophy were:

1. Ratios as follows:
  - A. Fresh heart weight to body weight at death
  - B. Fixed and prepared total ventricular weight to body weight at death
  - C. Fixed and prepared left ventricular weight to body weight at death
  - D. Fixed and prepared right ventricular weight to body weight at death
  - E. Fixed and prepared septal weight to body weight at death
  - F. Fresh auricular weight to body weight at death
2. Ratio of fresh heart weight to body area at death
3. Ratio of fixed and prepared left ventricular weight to right ventricular weight
4. Direct measurement of the diameters of cardiac muscle fibers, with the use of a filar micrometer, from
  - A. The apex of the left ventricle (paraffin sections)
  - B. The lower one fourth of the interventricular septum (paraffin sections)
  - C. Macerated, teased and stained specimens of the apex of the left ventricle

*Statistical Analysis.*—We made the following statistical computations:

1. Frequency polygons, showing for both inoculated and control series:
  - A. Ratio of heart weight to body weight at death
  - B. Ratio of fixed and prepared total ventricular weight to body weight at death
  - C. Ratio of fixed and prepared left ventricular weight to body weight at death
  - D. Ratio of fixed and prepared right ventricular weight to body weight at death
  - E. Ratio of fixed and prepared septal weight to body weight at death
  - F. Ratio of estimated fresh auricular weight to body weight at death
  - G. Average diameter of cardiac muscle fibers from paraffin sections of apex of left ventricle
  - H. Average diameter of cardiac muscle fibers from paraffin sections of lower one fourth of interventricular septum
  - I. Average diameter of cardiac muscle fibers from macerated specimens of apex of left ventricle
  - J. Average diameter of cardiac muscle fibers based on the sum of all microscopic muscle fiber diameter measurements
2. Scatter diagrams for both inoculated and control series between the following variables:
  - A. Ratio of fresh heart weight to body weight at death in relation to
    - a. Total exercise time in minutes
    - b. Ratio of fresh heart weight to body surface area

- c. Body weight at death
- d. Fresh heart weight
- e. Average muscle fiber diameter
- B. Total exercise time in relation to
  - a. Average muscle fiber diameter
  - b. Ratio of fresh heart weight to body surface area
- C. Ratio of fresh heart weight to body surface area in relation to
  - a. Fresh heart weight
  - b. Body surface area
  - c. Average muscle fiber diameter

3. Linear step-interval distribution between inoculated and control groups showing percentage deviation from average values of the same units in the control series:

- A. Ratio of fresh heart weight to body area at death
- B. Ratio of fresh heart weight to body weight at death
- 4. Pearson product-moment coefficient of correlation between:
  - A. Fixed and prepared total ventricular weight and body weight at death
  - B. Fixed and prepared left ventricular weight and body weight at death
  - C. Fixed and prepared left ventricular weight to right ventricular weight
  - D. Percentage of body weight lost during experimental period and ratio of fresh heart weight to body weight at death
- 5. The reliability coefficients of our data were computed by the method of

$$\frac{M_1 - M_2}{\sqrt{(\sigma_{M_1})^2 + (\sigma_{M_2})^2}} = \frac{D}{\sigma D}$$

and the obtained results interpreted on a table given by Garrett<sup>11</sup> for the following ratios:

- A. Fresh heart weight to body weight at death
- B. Fixed and prepared total ventricular weight to body weight at death
- C. Fixed and prepared left ventricular weight to body weight at death
- D. Fixed and prepared right ventricular weight to body weight at death
- E. Fixed and prepared left ventricular to right ventricular weight

6. The ratios of fresh heart weight to body weight for our seven normal dogs were calculated according to the previously described method

The raw data for inoculated, control and normal animals are presented in tables 1, 2, 3 and 4. The column numbers in table 1 represent the following values:

- 1. Identification number of dog
- 2. Sex of dog
- 3. Date of beginning of experimental period

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11. Garrett, H. E.: *Statistics in Psychology and Education*, New York, Longmans, Green & Co., 1926.

4. Body weight at beginning of experimental period
5. Total exercise time in treadmill in minutes
6. Running ability: 1 (best) to 4 (poorest)
7. Death during course (C) of, or end (E) of, experimental period
8. Date of end of experimental period
9. Length of experimental period in days
10. Body weight at end of experimental period
11. Percentage of original body weight lost during experimental period
12. Cause of death
13. Average diameter of muscle fibers—paraffin sections from apex of left ventricle—in microns
14. Average diameter of muscle fibers—paraffin sections from lower quarter of interventricular septum—in microns
15. Average diameter of muscle fibers—teased, macerated, stained fibers from apex of left ventricle—in microns
16. Estimated surface area of dog in square meters
17. Ratio of fresh heart weight at death to estimated area of body surface at death
18. Percentage deviation of the ratio of heart weight to surface area from the control average
19. Ratio of fresh heart weight at death to body weight at death
20. Percentage deviation of the ratio of heart weight to body weight from the control average
21. Ratio of heart weight (fresh) to body weight at beginning of experimental period, with heart weight at death taken as a standard
22. Ratio of heart weight to body weight at beginning of experiment, with normal (Herrmann<sup>9</sup>) heart weight taken as standard
23. Ratio of fixed and prepared left ventricular weight to right ventricular weight
24. Total weight of fresh heart at death in grams
25. Total weight of fixed and prepared heart in grams
26. Ratio of fresh heart weight to body weight at death
27. Total weight of fixed and prepared ventricles in grams
28. Total weight of fixed and prepared ventricles to body weight at death
29. Weight of fixed and prepared left ventricle in grams
30. Ratio of weight of fixed and prepared left ventricle to body weight at death
31. Weight of fixed and prepared right ventricle in grams
32. Ratio of weight of fixed and prepared right ventricle to body weight at death
33. Weight of fixed and prepared septum in grams
34. Ratio of weight of fixed and prepared septum to body weight at death
35. Estimated weight of fresh auricles in grams
36. Ratio of estimated weight of fresh auricles to body weight at death

## ESTIMATIONS OF CARDIAC HYPERTROPHY

*Method 1 A.*—The ratio of mean fresh heart weight to body weight at death of our inoculated series is 0.00883 with a  $\sigma$  of 0.000360; that of our control series, 0.00792 with a  $\sigma$  of 0.000336, and that of normal dogs, as reported by Herrmann,<sup>9</sup> 0.00798 with a  $\sigma$  of 0.0001036. Since the ratios of the control dogs, which were subjected to the same severe and prolonged exercise as the inoculated group, remained an average of but 0.9 per cent below reported normal level values, we are reasonably sure in concluding that the observed difference is due to unavoidable experimental errors, and that severe and prolonged exercise in the absence of toxic injury does not raise the ratio of heart weight to body weight. The inoculated group shows a gain of 10.08 per cent, which indicates a definitely demonstrable gross relative cardiac hypertrophy, according to this method.

*Method 1 B, C, D, E, F.*—The increased ratio of heart weight to body weight is chiefly due to the increased size of the left ventricle, with the septum, right ventricle and right auricle hypertrophied, as shown in table 5.

The figures for fixed normal auricles are not given by Herrmann,<sup>9</sup> but a normal average is estimated from reported figures for the purpose of this paper. From table 5 it may be seen that the septum and the left ventricle are increased the most, with an apparently smaller involvement of the right chambers of the heart. It is possible that our particular dissection of the right ventricle and septal portions might have included more heart muscle in the septal portion at the expense of the right ventricular weight, a condition that would explain the apparent disproportion between these figures. The ratios quoted, based on the weights of the fresh heart, fixed and prepared total ventricles and left ventricle, are unaffected by this factor and suggest a moderate gross relative cardiac hypertrophy according to this method of expression. These figures, in the main, corroborate the work of Stewart.<sup>5</sup>

*Method 2.*—On account of the fact that absolute weights of hearts are useless for comparison because of variations in body size, we are forced to relate the heart weights to some measure of body size. There is evidence<sup>12</sup> that, in general, the heart weight, as well as other organ weights, of a group of animals in a similar state of nutrition, but of varying size, shows less variability when expressed as the ratio of heart weight to body surface area than when expressed as the ratio of heart weight to body weight. To investigate this matter, we have calculated

12. MacKay, L. L., and MacKay, E. M.: Am. J. Physiol. **83**:179, 1927.

TABLE 1.—

Group		1	2	3	4	5	6	7	8	9	10	11	12
		Dog	Sex	Date Experiment Began	Body Weight When Experiment Began, Kg.	Total Exercise Time, Min.	Running Ability*	Death in Course of or at End of Experiment	Date Experiment Ended	Days of Experiment or of Life of Dog	Body Weight When Experiment Ended, Kg.	Weight Lost, per Cent	Cause of Death
Inoculated	1	M	12/ 3/27	8.2	2,145	12	C	6/30/28	209	6.8	16.2	Fighting	
	2	M	12/ 4/27	10.9	1,539	12	C	8/18/28	256	10.3	5.1	Fighting	
	3	M	12/ 4/27	14.3	1,035	12	C	2/12/28	70	11.1	22.3	Acute cardiac failure	
	5	M	12/ 4/27	11.8	912	12	C	2/24/28	82	7.9	32.7	Acute cardiac failure	
	7	M	12/ 8/27	11.3	2,911	12	C	8/12/28	247	9.3	18.0	Fighting	
	10	M	12/ 8/27	9.1	2,422	12	C	7/ 4/28	209	8.4	7.5	Fighting	
	16	M	1/10/28	12.4	392	4	C	2/24/28	45	7.7	38.2	Acute cardiac failure	
	22	M	7/27/28	9.3	16	3	E	2/23/29	154	9.1	2.5	Experimental	
	24	M	7/27/28	4.7	113	3	C	12/ 8/28	134	3.9	17.0	Fighting	
	8	F	12/ 8/27	8.1	3,462	3	E	11/ 1/28	329	6.6	19.5	Experimental	
	9	F	12/ 8/27	5.6	3,778	1	E	11/ 1/28	329	4.2	28.8	Experimental	
	17	F	1/14/28	5.0	835	2	C	4/ 3/28	82	3.4	31.7	Acute cardiac failure	
Average.....				9.28	1,547	2.54			179	7.41	19.9		
Control	6	M	12/ 4/27	15.2	3,861	1	E	11/ 1/28	333	13.2	13.5	Experimental	
	13	M	12/ 8/27	11.1	3,575	2	E	11/ 1/28	329	10.9	2.0	Experimental	
	20	M	7/27/28	7.0	2,200	2	E	2/23/29	210	8.2	+11.6	Experimental	
	25	M	7/27/28	7.7	1,271	2	C	1/11/29	167	7.5	3.0	Fighting	
	26	M	7/27/28	6.8	440	4	E	2/23/29	209	7.3	+5.0	Experimental	
	27	M	7/27/28	4.7	465	4	C	10/23/28	88	5.6	+12.2	Fighting	
	11	F	12/ 8/27	6.6	3,610	3	E	11/ 1/28	330	5.2	20.5	Experimental	
	14	F	1/ 9/28	5.9	603	4	E	11/ 1/28	293	4.8	19.1	Experimental	
	28	F	11/ 3/28	9.4	830	1	E	2/23/29	111	7.5	20.5	Experimental	
	29	F	11/ 3/28	13.0	837	1	E	2/23/29	111	12.5	4.0	Experimental	
	30	F	11/ 3/28	11.0	851	2	E	2/23/29	111	8.5	14.8	Experimental	
	31	F	11/ 3/28	14.2	829	1	E	2/23/29	111	14.1	1.0	Experimental	
Average.....				8.91	1,612	2.25			202	8.76	4.97		
Endocarditis control		12	M	12/ 8/27	10.0	792	4	C	3/26/28	169	8.2	18.1	Acute cardiac failure

TABLE 1.—General Data.—Continued

Group		Dog	24	25	26	27	28	29	30	31	32	33	34	35	36
			Heart			Ventricles		Left Ventricle		Right Ventricle		Septum		Auricle	
			Total Weight When Fresh (at Death), Gm.	Total Weight When Fixed and Prepared, Gm.	Ratio Fresh Heart Weight to Body Weight at Death	Total Weight When Fixed and Prepared, Gm.	Ratio Fixed and Prepared Total Weight to Body Weight at Death	Weight When Fixed and Prepared, Gm.	Ratio Fixed and Prepared Weight to Body Weight at Death	Weight When Fixed and Prepared, Gm.	Ratio Fixed and Prepared Weight to Body Weight at Death	Weight When Fixed and Prepared, Gm.	Ratio Fixed and Prepared Weight to Body Weight at Death	Weight When Fresh, Gm. (Estimated)	Ratio Fresh Weight to Body Weight at Death
Inoculated	1	57.1	52.0	0.00835	51.8	0.00757	24.0	0.00361	17.0	0.00248	10.8	0.00158	5.15	0.00075	
	2	78.5	75.9	0.00762	70.4	0.00684	34.0	0.00329	22.8	0.00221	13.6	0.00132	8.20	0.00079	
	3	77.2	72.1	0.00695	67.0	0.00604	36.7	0.00330	16.1	0.00245	14.2	0.00128	10.10	0.00091	
	5	83.6	53.2	0.01052	74.9	0.00945	38.0	0.00321	19.9	0.00274	17.0	0.00234	8.61	0.00118	
	7	67.4	64.2	0.00725	60.1	0.00646	28.4	0.00306	21.9	0.00236	9.8	0.00105	7.35	0.00078	
	10	69.0	68.4	0.00822	62.6	0.00746	29.9	0.00357	18.9	0.00226	13.8	0.00165	6.00	0.00073	
	16	59.2	39.3	0.00770	49.0	0.00636	26.3	0.00337	12.5	0.00160	10.2	0.00131	10.00	0.00128	
	22	77.5	82.5	0.00855	67.1	0.00740	36.6	0.00404	16.9	0.00186	13.6	0.00150	10.30	0.00114	
	24	49.0	42.0	0.01272	38.1	0.00978	17.4	0.00451	11.1	0.00288	9.6	0.00249	3.90	0.00104	
	8	50.0	54.8	0.00760	49.7	0.00756	25.5	0.00388	13.3	0.00202	11.0	0.00167	5.00	0.00076	
Control	9	43.5	46.5	0.01040	40.7	0.00974	19.5	0.00465	12.0	0.00286	9.3	0.00221	5.75	0.00137	
	17	34.1	27.0	0.01004	30.2	0.00888	14.8	0.00436	8.2	0.00243	7.1	0.00209	3.84	0.00113	
	Average.....		63.9	54.8	0.00883	55.1	0.00778	26.8	0.00391	15.9	0.00234	11.7	0.00171	7.01	0.00099
	6	106.0	96.8	0.00805	83.6	0.00635	42.8	0.00325	22.8	0.00173	18.5	0.00141	12.80	0.00097	
	13	84.1	76.3	0.00772	69.0	0.00633	34.3	0.00314	15.8	0.00145	19.0	0.00174	7.80	0.00071	
	20	61.1	67.5	0.00750	53.8	0.00660	26.3	0.00323	14.4	0.00177	13.1	0.00161	7.22	0.00085	
	25	66.0	57.8	0.00881	58.0	0.00775	33.7	0.00450	12.9	0.00172	11.4	0.00152	7.98	0.00106	
	26	60.5	65.0	0.00835	54.0	0.00744	23.2	0.00320	15.9	0.00219	14.9	0.00206	6.46	0.00089	
	27	40.6	38.0	0.00719	33.5	0.00593	18.3	0.00322	7.25	0.00128	8.0	0.00141	4.50	0.00079	
	11	35.6	32.4	0.00685	28.4	0.00545	14.6	0.00281	7.75	0.00149	6.0	0.00116	4.00	0.00072	
Endocarditis control	14	39.0	36.0	0.00818	30.3	0.00635	15.8	0.00320	7.25	0.00152	7.3	0.00152	5.75	0.00120	
	28	69.5	77.0	0.00929	62.2	0.00831	28.0	0.00374	18.9	0.00232	15.3	0.00205	7.22	0.00096	
	29	106.5	117.0	0.00855	93.9	0.00733	46.4	0.00373	25.5	0.00205	21.9	0.00176	12.70	0.00102	
	30	64.5	56.0	0.00759	58.9	0.00693	29.5	0.00458	15.0	0.00232	14.4	0.00224	5.75	0.00089	
	31	98.0	91.5	0.00698	83.1	0.00592	40.1	0.00285	22.4	0.00160	20.6	0.00147	8.40	0.00060	
	Average.....		69.3	67.6	0.00792	59.0	0.006765	29.4	0.00345	15.1	0.00181	14.2	0.00165	7.53	0.00087
	12	78.6	75.5	0.00965	71.1	0.00960	35.0	0.00429	19.5	0.00239	16.6	0.00204	7.60	0.00093	

\* 1 = best; 4 = poorest. † Paraffin sections. ‡ Paraffin sections from lower quarter.

General Data

13	14	15	16	17	18	19	20	21	22	23
Average Diameter of Muscle Fiber, Microns										
Apex Left Ventri- cle†	Interventricular Septum†	Apex Left Ventri- cle Teased, Alne- crated, Stained	Surface Area, Sq.M.	Ratio Fresh Heart Weight to Body Area at Death	Percentage Deviation of Ratio 17 from Control Average	Ratio Fresh Heart Weight to Body Weight at Death	Percentage Deviation of Ratio 18 from Control Average	Ratio Fresh Heart Weight to Body Weight at Start, with Heart Weight at Death Taken as Standard	Ratio Heart Weight to Body Weight at Start, with Normal Heart Weight Taken as Standard	Ratio Flxed and Pre- pared Left Ventricle Weight to Right Ven- tricle Weight
7.51	7.95	7.99	0.403	141.0	— 1.4	0.00835	— 5.5	0.00909	0.00951	1.41
7.60	7.49	7.33	0.530	145.0	— 2.8	0.00762	— 3.8	0.00775	0.00841	1.49
7.45	7.68	7.64	0.556	137.9	— 4.2	0.00695	—12.2	0.00895	0.01028	2.28
7.86	7.31	7.59	0.446	187.3	—30.1	0.01052	—32.8	0.01360	0.01185	1.91
7.19	7.44	7.44	0.488	138.1	— 4.0	0.00725	— 5.5	0.00885	0.00974	1.30
7.93	8.17	8.06	0.457	151.0	— 4.9	0.00822	— 3.8	0.00890	0.00862	1.58
7.56	7.33	7.25	0.435	136.6	— 5.1	0.00770	— 2.8	0.01228	0.01290	2.05
7.01	7.35	6.98	0.478	162.0	—12.6	0.00855	— 5.0	0.00875	0.00820	2.17
7.06	7.59	6.99	0.274	178.9	—24.3	0.01272	—60.6	0.01310	0.00961	1.57
7.29	7.57	6.83	0.393	127.1	—11.7	0.00760	— 4.1	0.00945	0.00991	1.93
7.32	7.36	7.50	0.291	149.5	— 3.9	0.01040	—31.4	0.01460	0.01120	1.63
7.13	7.05	7.00	0.253	134.3	— 6.3	0.01004	—26.7	0.01470	0.01170	1.80
7.41	7.53	7.38	0.417	149.3		0.00883		0.01057	0.01001	1.765
5.48	5.96	5.61	0.623	170.0	—18.1	0.00805	— 1.6	0.00930	0.00923	1.88
6.11	6.63	6.32	0.550	153.0	— 6.4	0.00772	— 2.5	0.00786	0.00815	2.18
6.78	6.78	6.47	0.453	134.8	— 6.4	0.00750	— 5.3	0.00646	0.00715	1.82
6.58	6.62	6.37	0.427	154.5	— 7.4	0.00881	—11.3	0.00909	0.00823	2.61
6.32	6.60	6.33	0.418	144.7	— 0.6	0.00835	— 5.4	0.00705	0.00760	1.46
6.25	6.45	6.00	0.355	114.4	—20.5	0.00719	— 9.2	0.00640	0.00715	2.52
7.36	7.23	7.30	0.336	106.0	—26.4	0.00685	—13.5	0.00861	0.01003	1.88
6.28	6.55	5.82	0.318	122.8	—14.6	0.00818	— 3.2	0.01021	0.00989	2.18
6.50	6.77	6.51	0.427	162.7	—13.0	0.00929	—17.3	0.01170	0.01003	1.48
6.18	6.78	5.85	0.601	176.2	—22.5	0.00855	— 8.0	0.00890	0.00832	1.78
6.51	6.40	6.51	0.465	138.8	— 3.5	0.00759	— 4.2	0.00890	0.00938	1.97
6.55	6.78	6.39	0.651	150.3	— 4.4	0.00698	—11.9	0.00705	0.00806	1.79
6.41	6.62	6.29	0.469	143.9		0.00792		0.00863	0.00860	1.964
7.31	....	....	.....	.....	.....	0.00965	.. ...	0.01180	0.00975	1.80

TABLE 2.—Diameters of Muscles Fibers from Hearts of Normal Dogs

	Dog A	Dog B	Dog C	Dog D
Apical*	6.85	6.65	6.95	6.00
	6.55	6.80	6.85	6.05
	7.10	6.95	6.85	5.95
	6.80	7.05	6.70	6.50
	6.20	6.45	6.50	5.45
	6.10	6.50	7.10	6.05
	7.05	6.20	7.05	6.20
	6.85	6.50	6.45	6.10
	6.80	6.70	6.65	6.45
	6.60	6.55	7.20	6.05
	6.65	6.75	7.30	6.20
	6.65	6.80	7.10	5.80
	6.35	6.45	7.00	5.90
	6.20	6.55	7.20	6.15
	6.50	6.65	6.60	6.30
	6.10	6.65	6.80	6.35
	6.45	6.75	6.85	5.45
	6.35	7.10	6.65	6.20
	6.70	6.60	6.70	5.85
	6.80	6.50	6.60	6.00
Average.....	6.58	6.66	6.86	6.05
Teased apical*	5.6	5.2	6.9	5.9
	6.6	6.3	6.8	5.8
	6.6	6.6	6.0	6.2
	6.7	6.7	7.1	6.1
	6.3	6.0	5.8	7.1
	6.1	5.6	7.3	6.1
	5.5	7.0	6.6	5.9
	6.2	6.2	5.9	6.0
	6.1	7.5	6.5	6.3
	6.4	6.6	6.3	6.2
Average.....	6.23	6.37	6.52	6.16

\* Diameters are expressed in microns.

TABLE 3.—*Diameters of Muscle Fibers from Hearts of Inoculated and Control Dogs*

	Inoculated Group of Dogs												Control Group of Dogs											
	1	2	3	5	7	10	16	22	24	8	9	17	6	13	20	25	26	27	11	14	23	29	30	31
Septal*	9.3	6.3	9.0	8.8	8.8	8.4	8.0	6.3	6.3	7.7	6.5	7.9	5.8	7.0	6.8	6.3	7.4	7.2	6.7	6.4	6.7	7.2	5.7	7.1
	8.1	8.1	7.3	7.3	8.4	8.7	6.9	6.8	7.8	6.8	8.6	7.6	6.4	6.1	7.6	5.4	5.8	7.1	7.2	6.5	7.3	5.4	5.1	6.8
	5.7	8.0	6.6	6.3	7.1	8.9	7.5	7.9	7.7	8.2	8.2	6.4	7.1	6.3	7.1	7.1	6.4	6.4	6.2	6.8	6.5	7.1	6.3	7.3
	8.0	7.3	8.0	7.8	6.9	8.3	7.5	8.4	7.9	7.5	7.2	6.1	6.1	7.2	5.6	6.6	6.5	5.6	7.3	7.2	7.2	6.3	5.6	7.4
	5.8	6.8	8.0	7.2	7.3	7.7	7.9	6.5	7.4	7.6	7.3	7.4	5.4	7.5	6.7	5.3	5.4	7.4	6.0	6.0	8.1	7.6	7.6	5.9
Septal*	9.9	6.5	6.2	6.8	7.0	9.3	6.7	7.6	7.0	7.1	7.4	7.2	5.9	5.9	6.8	6.2	7.3	6.1	6.2	6.3	6.5	7.3	7.1	7.6
	7.7	8.3	8.5	7.8	7.5	7.5	7.7	6.9	6.7	6.9	8.0	6.0	5.7	6.4	6.0	7.0	7.3	6.9	7.3	6.9	5.7	7.1	6.8	6.5
	8.3	8.2	8.4	7.0	6.9	7.8	7.0	6.3	8.7	8.9	8.7	6.9	5.6	6.5	6.7	6.5	6.7	6.0	7.6	6.1	6.3	7.5	7.3	7.1
	7.0	7.7	6.8	0.9	7.4	8.1	7.4	8.5	8.3	7.3	5.7	8.0	6.2	6.5	7.7	7.8	6.6	5.6	8.7	7.1	7.3	6.4	6.1	6.1
	9.7	7.7	8.0	7.2	7.1	7.0	6.7	8.3	8.1	7.7	6.0	7.0	5.4	6.9	6.8	7.0	6.6	6.2	8.1	0.2	6.1	5.9	6.4	6.0
Average.....	7.95	7.49	7.68	7.31	7.44	8.17	7.33	7.35	7.59	7.57	7.36	7.05	5.96	6.63	6.78	6.32	6.60	6.45	7.13	6.55	6.77	6.78	6.40	6.78
Apical*	9.5	5.8	8.4	7.8	7.3	7.1	7.5	7.2	6.6	7.1	6.4	7.0	4.8	5.7	5.1	6.4	6.2	6.2	6.2	6.0	6.0	6.4	5.2	7.2
	7.2	6.7	6.1	7.4	7.7	9.0	7.0	7.4	7.0	7.3	6.5	7.7	5.4	5.4	6.3	6.7	5.6	6.6	7.8	7.6	7.0	5.1	5.4	7.4
	7.5	6.5	6.8	7.9	8.0	8.7	8.1	6.2	6.4	7.3	8.7	6.9	5.9	6.5	6.5	7.4	7.0	6.2	8.7	4.5	5.4	5.8	7.2	6.8
	7.6	8.1	6.4	8.7	8.5	8.0	7.4	7.5	7.1	6.4	6.2	7.1	4.7	6.0	7.2	5.9	6.8	7.0	0.9	6.0	6.6	6.3	7.0	6.2
	6.1	8.0	6.7	7.5	6.9	8.6	7.6	7.3	8.3	6.8	7.5	7.0	5.9	5.0	7.5	7.3	6.6	0.8	6.8	6.5	5.7	6.0	7.6	6.4
Apical*	7.4	8.9	8.4	9.1	6.9	7.0	7.8	6.3	7.0	7.0	7.4	6.5	5.9	7.3	6.0	5.8	6.2	5.6	7.0	0.3	0.9	6.8	7.0	5.0
	7.1	9.0	8.7	6.8	7.4	6.1	6.9	7.1	6.2	7.7	6.8	8.2	5.3	7.0	7.2	6.2	6.0	6.8	7.5	5.9	5.9	6.2	0.2	7.5
	8.1	8.0	7.2	8.1	6.2	7.8	8.4	7.2	7.2	8.2	8.6	6.8	5.4	6.0	7.9	7.1	6.6	5.6	8.6	6.5	7.5	7.4	7.2	7.1
	8.2	7.6	7.4	7.7	6.3	9.4	8.2	7.0	6.9	6.9	6.5	7.5	5.1	5.9	6.7	6.3	5.6	5.4	6.3	6.1	6.9	5.6	6.2	5.6
	6.4	7.4	8.4	7.6	6.7	7.6	6.7	6.9	7.9	8.2	8.6	6.6	6.1	6.3	7.4	6.7	6.6	6.3	7.8	7.4	7.1	6.2	6.1	6.3
Average.....	7.51	7.60	7.45	7.86	7.19	7.93	7.36	7.01	7.06	7.29	7.32	7.13	5.48	6.11	6.78	6.58	6.32	6.25	7.36	6.28	6.50	6.18	6.51	6.55
Tense apical*	7.0	7.2	7.4	8.2	7.3	7.0	6.3	7.6	7.8	8.2	7.2	7.0	5.9	5.9	6.2	6.2	6.8	6.2	6.4	5.3	6.9	4.9	5.3	6.6
	8.4	7.0	8.5	7.8	8.1	8.3	7.0	7.9	6.0	6.8	7.6	6.6	5.4	6.1	7.3	6.2	6.3	7.0	6.7	5.7	5.8	5.7	6.9	7.1
	7.7	6.8	7.2	8.4	8.1	8.6	7.3	7.0	6.6	7.4	6.7	6.3	5.9	5.9	5.9	5.8	6.7	6.6	8.2	6.5	6.3	6.2	6.8	6.7
	7.3	7.0	7.7	8.4	7.8	8.5	6.9	6.9	6.9	5.9	7.3	7.8	5.4	6.6	6.7	5.8	6.1	6.1	7.5	6.1	6.5	6.2	7.1	6.7
	7.8	8.0	6.7	8.5	7.1	7.5	7.6	8.0	7.2	7.0	7.8	6.8	5.3	5.6	6.5	6.4	6.5	6.3	7.2	5.8	6.8	5.7	6.6	6.4
Tense apical*	7.9	7.6	8.2	6.6	7.0	8.2	6.7	6.5	7.5	6.0	8.0	6.6	6.1	6.5	6.7	6.6	6.3	5.1	6.9	5.1	6.1	5.9	7.2	6.1
	8.8	7.8	7.1	7.2	6.5	8.7	7.1	7.3	7.1	6.2	7.9	7.5	4.7	5.9	6.1	5.7	6.8	6.6	7.6	5.5	6.9	5.8	6.8	6.5
	9.4	7.6	7.4	7.0	7.6	7.3	8.0	6.1	6.9	7.1	8.1	7.1	6.4	6.9	6.8	7.6	5.6	5.3	7.4	6.8	6.6	7.1	6.0	5.9
	8.1	7.0	8.8	6.9	7.1	9.0	8.0	6.2	7.6	6.6	8.0	7.3	6.2	6.8	5.8	5.6	6.2	5.3	6.9	6.3	5.9	5.3	6.2	6.0
	7.6	7.3	7.4	6.9	7.8	7.5	7.6	6.3	6.5	7.1	7.2	7.0	4.8	7.0	6.7	7.3	6.0	5.5	8.2	5.1	7.3	5.7	6.2	5.9
Average....	7.99	7.33	7.64	7.59	7.44	8.06	7.25	6.98	6.99	6.83	7.58	7.00	5.61	6.32	6.47	6.37	6.33	6.00	7.30	5.82	6.51	5.85	6.51	6.39

\* Diameters are expressed in microns.

TABLE 4—Data on Heart Weight of Normal Dogs

Dog	Body Weight, Kg	Fresh Heart Weight, Gm	Ratio Fresh Heart Weight to Body Weight at Death
A	11.3	86.1	0.00761
B	6.3	46.2	0.00733
C	9.8	79.5	0.00810
D	7.5	58.5	0.00780
E	10.0	77.6	0.00776
F	9.2	72.0	0.0081
G	5.15	41.4	0.00802
Average	8.47	66.0	0.00776

body surface area for each of the dogs according to the formula<sup>13</sup> and have calculated the corresponding ratios of heart weight to body area:

$$\text{Body Area} = (\text{Body Weight})^{0.667} \text{ times } 0.112$$

Group	Body Area, Sq M	Ratio of Heart Weight to Body Area	Percentage Above Control
Inoculated	0.417	149.3	3.9
Control	0.469	143.9	

Percentage deviation of unit figures from control averages, as suggested by Dr. Thomas Addis,<sup>14</sup> are as follows:

Group	Ratio of Heart Weight to Body Area	Ratio of Heart Weight to Body Weight
Inoculated	- 2.96 per cent	+11.20 per cent
Control....	+ 0.38 per cent	- 0.51 per cent

These results indicate that the heart weight of the inoculated series is less variable when expressed as the ratio of heart weight to body area than when expressed as the ratio of heart weight to body weight, although the number of experimental animals used is too small to permit such a conclusion.

*Method 3*—The ratios of the weight of the fixed and prepared left ventricle to that of the right ventricle are found to be:

Group	Ratio of Left Ventricle to Right Ventricle	Percentage Above Normal
Inoculated	1.765 $\sigma$ 1.79	20.9
Control	1.964 $\sigma$ 1.99	34.2
Normal	1.461 $\sigma$ 0.317	

13 Rogers, C. G. Textbook of Comparative Physiology, ed. 1, New York, McGraw-Hill Book Company, 1927, p. 357

14 Addis, Thomas. Personal communication



Since the probable errors of the inoculated and of the control group are so large in comparison to the means of these values, relatively little credence can be placed in the apparent increase over the normal values other than as indicating a general tendency.

*Method 4 A, B, C.*—Direct measurement of the diameters of cardiac muscle fibers with the use of a filar micrometer and oil immersion lens on stained specimens gives evidence of a microscopic cardiac hypertrophy. The data are shown in tables 2 and 3. The results may be summarized as follows:

Group	Diameter of Muscle Fiber, Microns	Percentage of Normal
Inoculated.....	7.44	115.0
Control.....	6.45	99.9
Normal.....	6.465*	

\* Normal values are not obtainable in the literature. The value given here is from our series of normal dogs.

TABLE 5.—*Hypertrophy as Estimated by Method 1 B, C, D, E, F*

Ratio*	Group	Value of Ratio		Percentage of Normal	
Fixed and prepared tissue					
TV to BW.....	Inoculated	0.00786	σ	0.00068	124.0
	Control	0.00674		0.00070	106.0
	Normal	0.00635		0.00075	
LV to BW.....	Inoculated	0.00394	σ	0.000397	131.0
	Control	0.00345		0.00035	115.9
	Normal	0.00300		0.00028	
S to BW.....	Inoculated	0.00171	σ	0.000418	148.0
	Control	0.00165		0.000306	142.0
	Normal	0.00116		(Not reported)	
RV to BW.....	Inoculated	0.00235	σ	0.000238	111.0
	Control	0.00180		0.000183	85.0
	Normal	0.00212		0.00216	
Fresh tissue					
A to BW.....	Inoculated	0.00092	σ	0.00219	126.0
	Control	0.00087		0.000158	111.8
	Normal	0.00078		(Not reported)	

\* TV = total ventricular weight (fixed and prepared tissue).  
BW = body weight.  
LV = left ventricular weight (fixed and prepared tissue).  
RV = right ventricular weight (fixed and prepared tissue).  
S = septal weight (fixed and prepared tissue).  
A = auricular weight (fresh tissue).

Figure 1 shows the relative proportion of overlap between the frequency polygons for the inoculated and the control series. It is this factor that is responsible for the greatly increased accuracy of this method over the gross methods of expressing cardiac hypertrophy. The fibers from the septum were generally found to be slightly larger than those from the apex, a fact that may perhaps find its explanation in the observation that the ventricular walls of a majority of the hearts were definitely thinned to two thirds or one half of the normal thickness at the particular apical point selected for the specimens. The findings of Goldberger<sup>7</sup> are corroborated by this work with the exception of cell volume, which we are unable to determine, owing to the fact that the

present thought on cardiac muscle<sup>15</sup> considers it a syncytium, with the result that the length of the cardiac muscle fiber cannot be determined. The findings of Tangl<sup>8</sup> fail to find support in our results.

#### STATISTICAL COMPUTATIONS

*Computations 1 A, B, C, D, E, F.*—The major difference between the inoculated and the control group, when the cases are plotted against the ratios of total heart weight and of individual heart chamber weights

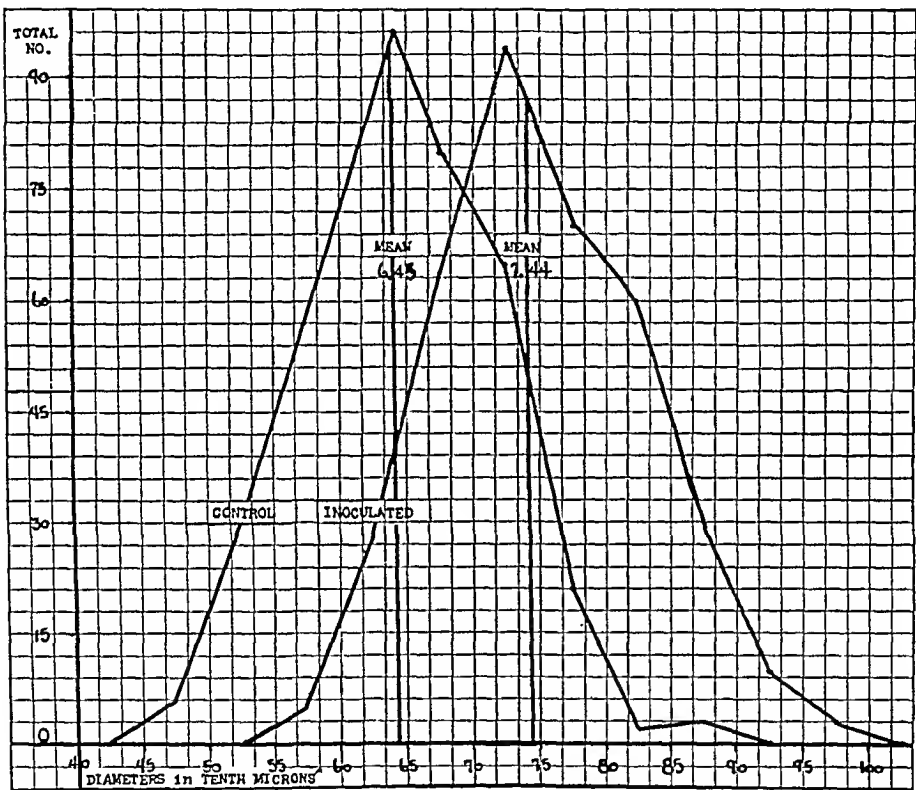


Fig. 1.—Individual muscle fiber diameters.

to body weight, consists of a skewness of the curves of the inoculated group to the higher ratio values, while the lower limits of the curves are roughly the same as those of the curves of the control group. This fact indicates that our ratios of cardiac hypertrophy based on body weight at death, and on the body area values derived therefrom, may perhaps be unduly influenced by the greater loss in body weight of the inoculated group. This would make such ratios, under our conditions, fail to express the degree of true cardiac hypertrophy actually present.

15. Strong, O. S., and Elwyn, A.: *Bailey's Textbook of Histology*, ed. 7, New York, William Wood & Company, 1925, p. 158.

Data on the heart weight at the beginning of the experiment are anatomically impossible to obtain, and even teleroentgenograms of dogs are inaccurate for the aforementioned estimations, with the result that we cannot, mathematically or statistically, estimate the relative effect of the greater loss in weight on these ratios. This matter will be further discussed under the head. Computation 2 Aa.

*Computations 1 G, H, I, J.*—The data relative to these computations have been previously discussed under the head, Estimations, Method 4 A, B, C.

*Computations 2 Aa.*—A moderate degree of negative<sup>16</sup> relationship between the ratio of fresh heart weight to body weight and the total number of minutes of exercise was found. The relationship is most marked in the inoculated group. This finding probably has its explanation in the observation that those dogs, dying of an intense toxemia, and therefore not living for the entire experimental period, as a general rule, showed the greatest ratios of heart weight to body weight.

*Computation 2 Ab.*—The ratio of heart weight to body weight and that of heart weight to body surface area show a positive correlation, indicating that these ratios express a number of common factors.

*Computation 2Ac.*—Control dogs show no apparent relationship between the ratio of heart weight to body weight, and body weight at death, while the inoculated series display a marked negative correlation. This fact suggests that the marked loss of body weight in the toxemic inoculated dogs may have had an undue influence on those dogs showing a remarkably high ratio of heart weight to body weight.

*Computation 2 Ad.*—A slight positive relationship was noted between the factors of the ratio of heart weight to body weight and gross fresh heart weight in grams.

*Computation 2 Ae.*—The average diameter of the muscle fibers, inasmuch as it is a single, definitely delimited measurement, is less subject to error than a ratio, in which a variation in either of its component factors affects the consequent ratio. The ratio of heart weight to body weight shows only a slight negative<sup>16</sup> relationship to the average muscle fiber diameter, and therefore we conclude that, at least under our particular experimental conditions, the ratio of heart weight to body weight is a less desirable method of expressing cardiac hypertrophy than the microscopic evidence afforded in increased diameter of the muscle fiber.

*Computation 2 Ba.*—In contrast to the findings in 2 Aa. we note that there is apparently no effect of prolonged exercise on the control

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16. "Negative" refers to correlation in the sense that an increase in one of the factors of the correlation is in general accompanied by a decrease in the other correlated factor.

dogs, with the result that the average diameters of their muscle fibers remain at practically the same general level regardless of running time. A very definite moderate degree of positive relationship is noted between the length of exercise time and the average diameter of the muscle fibers in the inoculated series. This shows that those dogs that survived the initial toxemia and continued exercise under the effects of a prolonged constant toxemia responded with a greater increase in the average diameter of the muscle fibers than those dying earlier in the experimental series.

*Computation 2 Bb.*—The ratio of heart weight to body surface area, when plotted against the total number of minutes of exercise, shows a scatter so great as to indicate practically no relationship.

*Computations 2 Ca, 2 Cb.*—A high degree of positive relationship is noted between the ratio of heart weight to body weight and its component factors in the control series. The inoculated group shows the same positive interrelationship to heart weight, but is variable when examined in comparison with body surface area in square meters.

*Computation 2 Cc.*—When the ratio of heart weight to surface area is considered in its relationship to the average diameter of the cardiac muscle cells of that particular animal, we find that the inoculated series shows a slight degree of positive relationship. This indicates that there is a smaller degree of common factors between the ratio of heart weight to body area and average diameter of muscle fibers than is shown between the ratio of heart weight to body weight and average diameter of muscle fibers. The control series displays a much more marked negative relationship. Under these circumstances, the slight positive relation shown by the inoculated group is probably a result of the chance combination of experimental variables, and therefore unreliable to use as a basis for conclusions.

*Computations 4 A, B.*—The Pearson product-moment coefficient of correlation between the total weight of fixed and prepared ventricles and body weight at death we find to be  $0.76 \pm 0.2$  for inoculated and  $0.90 \pm 0.2$  for control dogs. The same coefficients for the correlation between the weight of fixed and prepared left ventricle and body surface area are  $0.81 \pm 0.2$  for inoculated dogs,  $0.93 \pm 0.2$  for controls, and  $0.91 \pm 0.02$  for normal dogs. Since in all cases the value  $r$  is practically 4  $PE_r$ , it can be concluded that a definite positive relationship exists between these factors. The common factors in these measurements we find to be from 65 to 87 per cent of the possible total, and such percentages indicate a relatively high degree of elimination of avoidable experimental error in the entire series.

*Computation 4 C.*—The weight of the fixed and prepared left ventricles when compared with the weight of the fixed and prepared right

ventricles by means of a coefficient of correlation reveals an  $r$  of  $0.68 \pm 0.2$  for inoculated dogs,  $0.82 \pm 0.2$  for controls, and  $0.95 \pm 0.02$  for normal dogs. These figures show a definitely positive relationship, but of less degree than that shown by the ratios based on body weights. The common factors are estimated at a minimum of 49 per cent.

*Computation 4 D.*—The ratios of heart weight to body weight and the percentages of original body weight lost during the experimental series have been made the variable factors in a Pearson product-moment coefficient of correlation in order to determine whether the greater loss in body weight in inoculated dogs is responsible for the resultant ratios of heart weight to body weight. The following coefficients of correlation express the findings:

<sup>r</sup> Inoculated.....	$0.50 \pm 0.15$	25 per cent common factors
<sup>r</sup> Control.....	$0.31 \pm 0.18$	15 per cent common factors

An interpretation of these figures indicates that there is a slight positive correlation between the ratio of heart weight to body weight and the percentage of body weight lost, greater in the inoculated than in the control series. The uncommon factors are markedly predominant in these variables, and are in such excess that the common values bear little significant relationship to each other. Further calculations by Dr. Frank L. Griffin, professor of mathematics, Reed College, and by ourselves show that the increase in the ratio of heart weight to body weight can be more than accounted for by the greater percentage of body weight lost in the inoculated dogs. These calculations may be summarized as in table 6.

We consider it evident from the figures in table 6 that the greater ratio of heart weight to body weight of the inoculated dogs was due either to an actual decrease in the cardiac musculature during the experimental period or to the preponderant loss in body weight during the same time. The former is at variance with clinical findings, and therefore we assume that the greater percentage of body weight lost in the inoculated dogs affects the ratio of heart weight to body weight in such a manner as to render it relatively unreliable as a criterion of cardiac hypertrophy under our particular experimental conditions. The same conclusion may be applied to the ratios of heart weight to body surface area, as they are directly derived from the body weights at death. These conclusions do not deny that the ratios have value as estimates of cardiac hypertrophy, but to us they seem less reliable than other investigated methods.

*Computations 5 A, B, C, D.*—The reliability coefficients as shown in table 7 are self-explanatory, but are subject to the same criticism as discussed under the head, Computation 4 D. These figures illustrate the fallacy of depending on rough statistical data without a careful analysis

as to their meaning. The reliability coefficients indicate that a great deal of confidence may be placed in the ratio of heart weight to body weight and other similar ratios. This conclusion is at variance with the previously given evidence. In view of this fact it appears that if the ratios based on body weight at death were not unduly influenced by the variable percentage loss in body weight, the resulting ratios would be highly significant. Under our particular experimental conditions, these same ratios cannot be accepted as indicating nearly so great a degree of chance that difference is greater than zero as that actually shown, although as we have previously pointed out, a slight positive relationship is present. The ratio of left ventricular to right ventricular

TABLE 6.—*Summary of Calculations Showing That the Increase in the Ratio of Heart Weight to Body Weight Can Be Accounted for by the Greater Percentage of Body Weight Lost in the Inoculated Dogs*

Group	Probability That Heart Weight Is		Combined Probability for Entire Group That Heart Weight Is	
	Increased	Decreased	Increased	Decreased
Inoculated	0.06066	0.93934		
	0.16602	0.83398		
	0.06639	0.93361	$1.274 \times 10^{-14}$	$1.524 \times 10^{-2}$
	0.04006	0.95994		
	0.02500	0.97500		
	0.35569	0.64431		
	0.00071	0.99929		
	0.63683	0.36317		
	0.84850	0.15150		
	0.03593	0.96407		
	0.28774	0.71226		
	0.14007	0.85993		
	0.16354	0.83646		
Control	0.34458	0.65542	$1.560 \times 10^{-8}$	$1.191 \times 10^{-3}$
	0.64803	0.35197		
	0.70884	0.29116		
	0.77637	0.22363		
	0.53586	0.46414		
	0.00734	0.99266		
	0.10749	0.89251		
	0.59095	0.40905	$1.190 \times 10^{-3}$	
	0.07214	0.92786	$1.560 \times 10^{-8}$	
	0.15150	0.84850		

weight is uninfluenced by these factors, and represents a more nearly true value of the amount of relationship actually present. A chance selection, with equal probability of being true or false, would in itself result in a 50/100 selection.

*Computation 6.*—A comparison of the average ratios of heart weight to body weight of our series of normal dogs and those reported by Herrmann<sup>9</sup> is as follows:

Normal Group		Ratio of Heart Weight to Body Weight	
Ours.....		0.00776	$\sigma$ 0.000276
Herrmann's.....		0.00798	$\sigma$ 0.0001036

These figures, for a group of but seven dogs in comparison with the larger series of two hundred dogs, show that we have approximated the reported series in our group with a reasonable degree of accuracy.

On the basis of this check, we concluded that the normal values quoted by Herrmann<sup>9</sup> are a satisfactory series, and we therefore discontinued our series. The normal values quoted are from Herrmann unless there is a specific mention to the contrary.

*Computation 7.*—Column 7 in table 1 gives an estimation of the general running ability according to a four-unit graded system of recording. This was done in order that we might know whether the amounts

TABLE 7.—*Reliability Coefficients*

	Ratio of				
	Heart Weight to Body Weight	Total Weight Fixed and Prepared Ventricle to Body Weight	Left Ventricle Weight to Body Weight	Right Ventricle Weight to Body Weight	Left Ventricle Weight to Right Ventricle Weight
Inoculated					
Average.....	0.00883	0.00778	0.00391	0.00234	1.765
Probable error.....	0.000242	0.000458	0.000268	0.000160	1.21
Control					
Average.....	0.00792	0.00675	0.00345	0.00181	1.964
Probable error.....	0.000226	0.000532	0.000236	0.000123	1.34
Normal					
Average.....	0.00793	0.00635	0.00300	0.00212	1.461
Probable error.....	0.0000698	0.000505	0.000189	0.000146	0.214
Normal and control					
Diff *.....	0.00006	0.00040	0.00045	0.00031	0.503
PE diff †.....	0.000236	0.000225	0.000302	0.000191	1.36
Diff/PE diff ‡.....	0.254	1.78	1.49	1.62	0.37
Chances.....	57 to 100	88 to 100	84 to 100	86 to 100	60 to 100
Normal and inoculated					
Diff.....	0.00085	0.00143	0.00091	0.00022	0.304
PE diff.....	0.000253	0.000216	0.000328	0.000216	1.23
Diff/PE diff.....	3.36	6.62	2.86	1.02	0.247
Chances.....	99 to 100	99 to 100	97 to 100	75 to 100	57 to 100
Control and inoculated					
Diff.....	0.00091	0.00103	0.00046	0.00053	0.199
PE diff.....	0.000331	0.000222	0.000350	0.000202	1.81
Diff/PE diff.....	2.75	4.64	1.31	2.62	1.10
Chances.....	97 to 100	99 to 100	81 to 100	96 to 100	77 to 100

\* Diff = numerical difference between averages.

† PE = probable error of averages. Limits within which, plus and minus from the average, 50 per cent of the variables are found.

‡ PE diff = a calculated value (see reference) representing the variability of the two compared units, and therefore the expected mathematical variability.

§ Diff/PE diff = actual numerical difference between averages divided by the expected variability. It represents the amount of chance elements in the two variable series, zero representing all correlation being due to chance and infinity representing measurement of identical units.

of exercise represented by the figures for total exercise time in the two groups were approximately equivalent. Using 1 as the unit for the best running ability and 4 as that for the poorest, we find the average to be, inoculated, 2.54, and control, 2.25. These average values indicate that the groups are largely equivalent to each other in running ability, with a majority of the dogs running satisfactorily. The poorer running ability in the inoculated series, while relatively slight, has its most logical explanation in the toxicity of this group, with the consequent cardiac embarrassment during periods of exertion.

*Computation 8.*—Columns 21 and 22, table 1, represent an unsuccessful attempt to estimate the ratio of heart weight to body weight at the beginning of the experimental period by using (1) the animal's own fresh heart weight at death as a normal, on the assumption that of any single values this represents the best obtainable estimate of cardiac weight at the beginning of the period, and (2) an average normal heart weight as reported by Herrmann.<sup>9</sup> As no conclusions can be drawn from the resultant figures, we discontinued the consideration of these ratios.

#### COMMENT

The inoculated dogs were more or less depressed or toxic throughout the period of the experiment. They showed more fatigue than the non-

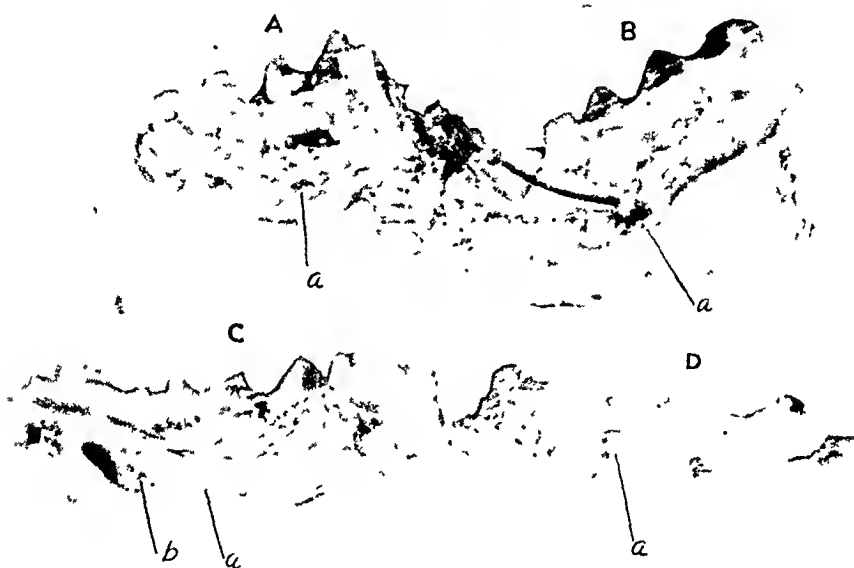


Fig 2—Gross jaw dissections from inoculated dogs: *A*, lower jaw of dog 16, showing an apical dental abscess (*a*); *B*, lower jaw of dog 1, showing an apical dental abscess (*a*); *C*, lower jaw of dog 17, showing an apical dental abscess (*a*) and a necrotic periodontal membrane (*b*); *D*, lower jaw of dog 9, showing an apical dental abscess eroding the outer table of the jaw (*a*).

inoculated dogs during the periods of exercise. Artificial respiration was resorted to eight times in the case of the inoculated dogs, whereas at no time was it necessary with the control animals. Only 25 per cent of the inoculated dogs lived through the experimental time, in comparison with 83.5 per cent of the control series. Both deaths in the latter series resulted from fighting, while in the former group four dogs died from acute cardiac failure. The average loss in weight in the control series was 58 per cent, in comparison with 19.9 per cent among the inoculated dogs. There is, therefore, considerable clinical evidence, as



well, that the inoculated dogs were made chronically ill by reason of their dental infection. The average duration of life among them was one hundred and seventy-nine days, whereas in the control group it was two hundred and two days, and ten of these dogs were killed at the end of the experimental time. The six months' experimental period represents roughly one twentieth of the average lifetime of dogs, which in turn corresponds to about three years of a human lifetime. One cannot deny a certain suggestive similarity in clinical course and pathologic lesions between these inoculated dogs and human patients suffering from those myocardial changes that are believed to follow, at times, especially in the wake of chronic dental and chronic sinus infection.

*Postmortem Observations.*—The observations at autopsy in the inoculated series of dogs were briefly as follows:

- Dog 1. Small dental abscess; very small vegetative mitral endocarditic lesion; no further lesions
- Dog 2. Moderate dental abscess; small vegetative mitral and aortic endocarditic lesions; no further lesions
- Dog 3. Small dental abscesses; moderate vegetative mitral and aortic endocarditic lesions; no further lesions
- Dog 5. Large dental abscess; moderate mitral and aortic verrucose endocarditic lesions; bilateral hilar tuberculosis; terminal bronchopneumonia; acute cardiac dilatation
- Dog 7. Small dental abscess; moderate mitral and aortic vegetative and verrucose endocarditic lesions; tapeworm; no further structural changes
- Dog 10. Moderate dental abscess; very small mitral endocarditic lesions; no further lesions
- Dog 16. Moderate dental abscess; moderate mitral and aortic verrucose endocarditic lesions; ulcerative colitis; acute cardiac dilatation
- Dog. 22. Small dental abscess; moderate aortic verrucose endocarditic lesion; mange; tapeworm
- Dog. 24. Small dental abscess; moderate verrucose and vegetative mitral and aortic endocarditic lesions; multiple abscesses in pectoralis majoris and minoris and rectus abdominis
- Dog 8. Small dental abscess; small mitral endocarditic lesion; tapeworm
- Dog 9. Moderate dental abscess with erosion of outer table of mandible; small mitral and aortic vegetative endocarditic lesion; no further structural changes
- Dog 17. Moderate dental abscess; small mitral endocarditic lesion; terminal bronchopneumonia; acute cardiac dilatation

The observations at autopsy in the control series were as follows:

- Dog 6. Emaciation
- Dog 13. Emaciation
- Dog 20. No gross structural changes
- Dog 25. Terminal bronchopneumonia; death by fighting
- Dog 26. Mange; tapeworm
- Dog 27. Death by fighting; evisceration; tapeworm
- Dog 11. Moderately thin

- Dog 14. No gross structural changes
- Dog 28. No gross structural changes
- Dog 29. No gross structural changes
- Dog 30. No gross structural changes
- Dog 31. No gross structural changes
- Dog 12. Control dog with endocarditis. No dental abscesses; moderate vegetative aortic and mitral endocarditic lesions; ulcerative colitis; no demonstrable primary focus of infection noted

Dog 12 was carried in the control series until autopsy was performed. No dental abscesses were seen, and no primary focus of infection could be identified at necropsy. The causative organism was unknown. This dog showed signs of toxicity during its life. The ratio of heart weight to body weight showed an apparent cardiac hypertrophy with a value of 0.00965. The average diameter of the apical cardiac muscle fibers of this dog was 7.31 microns. It was not included in either of our series on account of the unknown nature of the causative organism.

The histologic examination of the myocardium of the inoculated animals showed from a mild to a moderate grade of patchy parenchymatous degeneration of the individual fibers, which were observably increased in transverse diameter. Individual cell nuclei were found that showed irregularity, pyknosis, a deeper concentration of the chromatin and in some cases a partial or a complete doubling. A slight diffuse round cell infiltration by lymphocytes was seen in the majority of the sections. The cells were located for the most part perivascularly. Plasma cells were occasionally seen. A slight degree of fatty change, most marked subendocardially, was noted in dog 17. Multiple small focal hemorrhages were noted in the myocardium of dog 3. No abnormal changes were seen in the myocardium in any of the control or normal dogs. The heart muscle of dog 12 was accidentally not examined.

Many sections were stained and examined for micro-organisms within the tissue, but no organisms could be found. There are, seemingly, three possible explanations for the myocardial changes. The patchy areas of degeneration may have been produced by micro-organisms still present in the areas, but not recognized. The micro-organisms, on the other hand, may have been present in the tissues and been destroyed by the antibacterial properties of the heart muscle. Or, again, the myocardial changes may have been the result of toxins alone, and the blood stream have been sterile at all times. Blood cultures were made three times from inoculated dogs, but always were sterile.<sup>17</sup>

*Reliability of Results.*—Although the experimental groups were limited to twelve dogs in each series, in order that the experiment might not be too cumbersome and unwieldy, the results received therefrom appear

17. Dr. E. C. Rosenow prepared and studied sections from blocks of these tissues and corroborated these findings.

to be relatively reliable. The experimental error is estimated to be not over 1.5 per cent on an average. The methods have apparently measured from 49 to 87 per cent of the possible factors concerned in the problem, as noted under the heading, Computation 4 A, B. Thus the results can be considered to indicate that increasing experimental errors have been avoided and the unavoidable reduced to a minimum. It appears, therefore, that our twenty-four experimental animals comprise a population sufficiently large for the purpose of this study.

#### SUMMARY

Dental abscesses were demonstrated in all inoculated dogs. There were no other constant extracardiac structural changes.

The hearts of inoculated dogs constantly showed very small vegetative or verrucose mitral and (or) aortic endocarditic lesions, patchy parenchymatous degeneration, nuclear changes, increase in the diameter of the muscle cell, and a slight round cell infiltration.

A positive relationship between experimentally produced focal dental infection and cardiac hypertrophy as measured by diameters of muscle cells was noted.

Direct measurement of diameters of cardiac muscle fibers, under our experimental conditions, is a more reliable criterion of cardiac hypertrophy than ratios of heart weight to body weight or of heart weight to body surface area.

Stress and strain in the absence of focal infection did not affect the gross or the microscopic characteristics of the heart.

## EXPERIMENTAL EDEMA AND LIPEMIA PRODUCED BY REPEATED BLEEDING \*

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In 1928, Leiter <sup>1</sup> produced edema in dogs by bleeding them, separating the plasma from the red blood cells, making up the original volume of blood with Locke's solution and then reinjecting the resulting suspension of blood cells (plasmapheresis). The result was a striking depletion of plasma protein. His impression at the time was that the edema was associated with a low plasma protein, 3 per cent or less.

Barker and Kirk <sup>2</sup> were able to produce edema in dogs by removing the plasma proteins in essentially the same manner. It was their impression that edema was associated with a low plasma protein, 0.8 per cent or less. The kidneys of their dogs showed some tubular destruction, plasma cell and round cell infiltration and some increase in interstitial connective tissue. There was a moderate accumulation of fat in the tubules. Some glomeruli showed atrophy and occasional hyalinization of Bowman's capsule. These workers considered all these renal changes secondary to the prolonged hypoproteinemia. The urine from time to time showed albumin and casts. The plasma cholesterol level was rather irregular and at times moderately increased. The blood volume remained about the same during the period of edema. They concluded that the experimental disease in dogs might be the same as human lipoid nephrosis.

In a complete summary of his work on experimental edema in dogs secondary to plasmapheresis, Leiter <sup>3</sup> again stated that the edema was due to a low concentration of plasma protein, 3 per cent or less. He laid no particular stress on the concentration of plasma albumin. The cholesterol level was irregular, but never greatly or consistently elevated. The kidneys of his dogs showed infarcts and localized areas of sup-puration in the cortex. These the author explained as being secondary to emboli originating in the heart and due to lack of asepsis during the punctures of the heart. He also encountered the lesions described by Barker and Kirk and considered them as cases of spontaneous contracted kidneys in the dogs. All the edema fluids were low in protein

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\* Submitted for publication, Sept. 22, 1931.

1. Leiter, L.: Proc. Soc. Exper. Biol. & Med. **26**:173, 1928.

2. Barker, H. M., and Kirk, E. J.: Arch. Int. Med. **45**:319, 1930.

3. Leiter, L.: Arch. Int. Med. **48**:1, 1931.

(from 0.02 to 0.47 per cent). Leiter concluded that while the edema was doubtless of the nephrotic type, the experimentally induced state in the dog was not nephrosis.

Shelburne and Egloff<sup>4</sup> were able to produce edema in one dog by giving it a diet low in protein but adequate in carbohydrate, fat and water. The chemical, pathologic and clinical observations in this dog were essentially the same as those in two other dogs in which the edema was produced by plasmapheresis. On the whole, their results were in agreement with those of Barker and Kirk; the results differed in that the kidneys of their dogs showed only an accumulation of fat in the tubules; the urine also was free from albumin and casts. They were able to demonstrate glomerular lesions of various kinds in so-called normal dogs. In addition, they showed that with a given low concentration of plasma protein the amount of edema could be increased by giving sodium chloride and sodium bicarbonate. The edema fluids in their dogs were low in protein. They agreed with Leiter that the experimental disease in dogs is not nephrosis.

The purpose of my experiments was to determine whether edema could be produced simply by bleeding an animal repeatedly over a prolonged period. During the course of the work, the changes in the plasma proteins and lipoids were noted. The kidneys of all the animals and the livers of two were likewise analyzed for their lipid content.

#### EXPERIMENTAL PROCEDURE

About thirty well nourished adult male rabbits were selected and kept under observation for at least one month before any work was undertaken. The fifteen rabbits finally selected showed hemoglobin values over 100 per cent and red cell counts over 7,000,000. The white cell count never exceeded 8,000. The urine was normal on repeated examinations. The diet consisted of carrots, parsnips, alfalfa hay, corn and water.

The work was carried out at an altitude of approximately 4,000 feet (1,219.2 meters) above sea level and at a barometric pressure of about 640 mm. of mercury.

The rabbits were bled at intervals of from 36 to 45 hours; from 40 to 75 cc. of blood was removed each time from the right ventricle of the heart. This was done under local anesthesia, with a long, thin needle.

The blood volume was determined by the method of Keith, Rowntree and Geraghty.<sup>5</sup>

The plasma proteins were fractionated and precipitated by the method of Howe.<sup>6</sup> The total protein nitrogen and the albumin nitrogen were determined by the micro-Kjeldahl technic of Berglund.<sup>7</sup> The procedure of Folin and Wu<sup>8</sup> was used to calculate the nonprotein nitrogen.

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4. Shelburne, S. A., and Egloff, W. C.: *Arch. Int. Med.* **48**:51, 1931.

5. Keith, N. M.; Rowntree, L. G., and Geraghty, J. T.: *Arch. Int. Med.* **16**:547, 1915.

6. Howe, P. E.: *J. Biol. Chem.* **49**:109, 1921; **57**:235, 1923.

7. Berglund, H.: Personal communication.

8. Folin, O., and Wu, H.: *J. Biol. Chem.* **38**:81, 1919.

TABLE 1.—*Rabbit 1*

Date	Amount of Blood Taken, Cc.	Hemo- globin	Erythro- cytes	Leuko- cytes	Urine	Total Blood Volume, Cc.	Plasma Cc.	Plasma Fats, Mg. per 100 Cc.		Total Plasma Protein, per Cent per Cent 100 Cc.	Plasma protein		Comment
								Saponi- fable	Nonsa- ponifiable		Albumin, min.	Glob. Nitrogen, ulin, Mg. per Cent 100 Cc.	
1/ 5/31	50	105	7,650,000	7,200	—	150	121	683	82	7.86	6.80	1.06	47
1/ 8/31	40	...	...	...	—	...	...	756	80	8.06	4.73	3.33	26
1/10/31	40	...	...	...	—	...	...	801	80	7.20	5.21	1.96	48
1/13/31	30	...	...	...	Alb. +	116	119	988	81	5.05	2.91	2.11	...
1/16/31	50	70	1,200,000	11,000	Alb. +	...	...	516	96	...	...	...	...
1/19/31	85	...	...	...	—	...	...	672	101	9.28	...	...	...
1/21/31	50	...	...	...	—	140	110	640	201	...	2.81	...	37
1/22/31	40	65	3,020,000	13,100	Alb. +	...	...	1,140	410	5.10	2.57	2.53	34
1/25/31	40	...	...	...	—	...	...	1,209	302	5.16	3.12	2.01	31
2/ 3/31	50	85	5,388,000	8,000	—	152	122	682	102	4.51	...	...	38
2/ 4/31	50	...	...	...	Alb. +	...	...	514	274	4.96	2.58	2.38	27
2/ 6/31	50	60	...	...	—	...	...	666	144	4.56	...	...	40
2/ 8/31	50	...	...	...	Alb. +	110	85	1,782	512	7.67	2.66	5.01	44
2/10/31	45	45	3,744,000	7,400	—	...	...	263	64	5.15	2.57	2.58	35
2/12/31	45	45	3,164,000	10,000	Alb. +	...	...	2,094	108	5.74	2.39	3.35	38
2/14/31	50	39	3,200,000	7,600	—	...	...	...	...	5.48	2.13	3.35	71
2/16/31	60	30	...	...	Alb. +	102	80	...	...	5.20	2.29	2.91	89
2/18/31	65	20	1,650,000	5,600	Alb. +, casts	...	...	1,404	79	5.09	1.87	3.22	93

Slight edema of serotum  
Edema of serotum  
++; bleeding discontinued  
No edema  
No edema  
No edema  
No edema  
Slight edema of serotum  
Edema of serotum  
++  
Edema of serotum  
++  
Edema of serotum and anterior abdominal wall  
+++; edema of hind legs ++; ascites

An ether-alcohol-ether extract was used in the analyses of the plasma lipoids. Ten cubic centimeters of plasma was dried and then extracted for three hours with warm ether. This was followed by extraction for one hour with boiling alcohol. The sample was then extracted for one hour with warm ether. The combined extracts were dried to a constant weight in a vacuum oven.

The total extracts were then separated into saponifiable (neutral fat) and non-saponifiable (sterol) fractions. The saponification was carried out by boiling the sample under a reflex condenser with alcoholic potassium hydroxide for one hour. The alcoholic potassium hydroxide was then evaporated on a steam bath and the resulting residue extracted for twelve hours with cold ether. This ether extract was designated as the unsaponifiable or sterol fraction.

Tissues (kidney and liver) and samples of urine were subjected to exactly the same procedure in the determination of their total lipid, saponifiable and non-saponifiable contents.

### RESULTS

*Rabbit 1* (table 1).—In rabbit 1, an anemia and a milky plasma with a true increase in fat developed after the third bleeding. The plasma continued to be milky, but the actual lipid content on one occasion fell to nearly the normal level. The plasma lipid level rose sharply just when the animal showed signs of edema.

A slight edema of the scrotum was noted on the seventeenth day. This rapidly increased within the following four days. The concentration of the total plasma protein fell slightly (5.10 per cent), while the concentrations of the albumin and globulin were about equal. On the twentieth day, bleeding was discontinued for one week. The edema and the lipemia rapidly subsided, and the plasma became clear.

Bleeding was then resumed, and the edema of the scrotum promptly returned on the fifth day. The plasma again became milky, and the total plasma lipid was greatly increased. The rise was always most marked in the neutral fat fraction. The concentration of the total plasma protein was practically the same as before (5.74 per cent), but that of the albumin had dropped to 2.39 per cent and that of the globulin had increased to 3.35 per cent. The albumin-globulin ratio continued to be reversed throughout the remaining lifetime of the animal.

The edema soon extended to the anterior abdominal wall, and at the same time ascites could be demonstrated. The animal now walked with difficulty. On the day before death there was a moderate edema of the hind legs. The plasma lipid level continued to be high, but the milky appearance disappeared several days before death. The animal lost about a pound in weight and became rather emaciated in spite of the massive edema of some of its tissues. There was a marked secondary anemia at all times. The total blood volume and the plasma volume fell as the bleeding progressed. The total nonprotein nitrogen (plasma) began to increase about four days before death and finally rose to 93 mg. per hundred cubic centimeters.

Fat could always be found in the urine after the lipemia was well established. It showed practically the same saponifiable and nonsaponifiable values as did the plasma fat. A small amount of albumin appeared in the urine from time to time. Numerous red blood cells, white blood cells and some granular casts were found in the urine on the day preceding the animal's death.

A summary of the data shows that the rabbit survived for forty-six days and lost 810 cc. of blood. This represents an approximate loss of 29 Gm. of albumin and 22 Gm. of globulin. The total amount of plasma protein lost amounted to 51 Gm.

**Postmortem Examination:** The following observations were made: marked edema of scrotum and anterior abdominal wall; massive ascites; moderate hydrothorax; no hydrocele; moderate subcutaneous edema of hind legs; moderate fatty metamorphosis of liver; surface of kidneys smooth and golden yellow; renal medulla pale; renal cortex golden yellow and all markings lost; remaining viscera pale; only trace of blood in large vessels; bone marrow yellow; great decrease in subcutaneous, mesenteric and perirenal fat.

**Microscopic Examination:** Sections of the kidney stained with hematoxylin-eosin were normal. Fresh frozen sections stained with sudan III showed some overstaining of the cytoplasm of the convoluted tubules, but no fat droplets. Fresh frozen sections of the liver, stained with sudan III, showed the cytoplasm of the cords moderately filled with fat droplets.

*Rabbit 2* (table 2).—In this animal a secondary anemia and lipemia were slow in developing. The increase in plasma fat was not nearly so marked as in rabbit 1, although the plasma was at times quite milky. The rise was always most marked in the neutral fat fraction. The concentration of the total plasma protein fell very little. The concentration of albumin was, however, moderately decreased, while that of globulin was increased. There was no rise in nonprotein nitrogen. The total blood volume and the plasma volume were decreased as the bleeding was continued.

After the lipemia was well established, the urine showed a moderate amount of fat. The saponifiable and nonsaponifiable values were about the same as those found in the plasma. At times there was a small amount of albumin in the urine.

The animal never showed edema. As the bleeding progressed, the anemia became very marked, there was a great loss of weight, and the rabbit became very emaciated. There was a watery diarrhea for several days before death.

A summary of the data shows that the animal survived the bleeding for thirty-five days and lost 782 cc. of blood. During this time,



TABLE 2.—*Rabbit 2*

Date	Amount of Blood Taken, Ce.	Hemo- globin	Erythro- cytes	Leuko- cytes	Urine	Total Blood Volume, Ce.	Plasma Ce.	Plasma Fats, Mg. per 100 Ce.			Total Plasma Protein, per Cent per Cent	Plasma Albu- min, per Cent	Plasma Glob- ulin, per Cent	Non- protein Nitrogen, Mg. per 100 Ce.	Comment
								Saponi- fable	Nonsa- ponifiable	Total					
1/13/31	37	107	7,200,000	7,200	—	150	121	698	78	776	7.20	5.76	1.44	38	7½
1/16/31	50	...	.....	.....	—	...	...	698	93	791	7.02	4.96	2.05	40	...
1/19/31	50	...	.....	.....	—	...	...	514	360	874	8.89	....	....	39	7
1/21/31	30	...	.....	.....	Alb. +	141	114	781	120	901	6.03	2.42	3.61	34	...
1/23/31	45	83	.....	.....	—	...	...	721	118	839	5.43	2.29	3.14	49	7
2/ 2/31	50	80	3,720,000	8,600	Alb. +	141	120	722	134	856	9.65	2.56	7.09	51	7
2/ 4/31	50	70	.....	.....	—	...	...	803	109	1,012	9.17	2.76	6.41	32	...
2/ 6/31	50	65	.....	.....	—	...	...	1,010	81	1,091	5.78	....	....	36	6½
2/ 8/31	50	50	.....	.....	—	...	...	1,256	76	1,332	5.76	2.99	2.76	35	6½
2/10/31	50	40	2,200,000	9,600	Alb. +	...	...	1,376	100	1,476	6.61	3.17	3.44	45	6
2/11/31	50	30	1,232,000	21,000	—	130	108	928	76	1,004	7.00	2.36	5.24	34	0
2/12/31	30	23	1,200,000	15,000	Alb. +	...	...	735	205	960	8.13	....	....	39	5½
2/14/31	50	30	.....	.....	Alb. +	...	...	706	112	818	6.88	2.56	4.32	35	5½
2/15/31	50	30	740,000	21,000	Alb. +	...	...	742	72	814	5.97	2.08	3.89	32	5
2/16/31	65	36	.....	.....	Alb. +	100	86	1,250	84	1,334	7.69	....	....	40	5
2/17/31	75	25	832,000	15,000	Alb. +	...	...	1,537	155	1,692	6.80	2.26	4.54	39	4¾

approximately 23 Gm. of albumin and 32 Gm. of globulin were removed. The total amount of plasma protein lost amounted to 55 Gm.

**Postmortem Examination:** The observations were as follows: great emaciation; no edema; practically no subcutaneous mesenteric or perirenal fat; all tissues pale; moderate fatty metamorphosis of liver; surfaces of kidneys smooth; renal medullae pale and cortices golden yellow; all markings obliterated; bone marrow yellow, except at extreme ends.

**Microscopic Examination:** Sections of the kidneys stained with hematoxylin-eosin were normal. Fresh frozen sections stained with sudan III showed some overstaining of the cytoplasm of the convoluted tubules, but no fat droplets. Fresh frozen sections of the liver, stained with sudan III, showed the cytoplasm of the cords moderately filled with fat droplets.

*Rabbit 3* (table 3).—This animal was the most resistant one of the group. A secondary anemia developed rapidly, but never became as severe as in rabbits 1 and 2, even though more blood was lost. The lipemia was slow in developing, but once it was established it became the most marked of any. During the last twenty-one days of the animal's life, the total plasma fat was generally over 2,000 mg., and at one time it rose to over 4,800 mg. per hundred cubic centimeters. This striking increase was always most marked in the neutral fat fraction. The concentration of the total plasma protein showed little reduction. At times it rose to over 8 and 9 per cent. The concentration of albumin fell rapidly and at one time was only 1.61 per cent, the lowest concentration encountered in any one of the four rabbits. The globulin concentration was simultaneously increased. The plasma nonprotein nitrogen was never increased. The total blood volume and the plasma volume were decreased as the bleeding progressed.

The urine began showing large amounts of fat several days after the onset of the lipemia. This was mostly neutral fat. At times there was a small amount of albumin, and on one occasion, a few granular casts

The rabbit never became edematous. There was considerable loss in weight, and the animal became listless and greatly emaciated. There was a watery diarrhea for about one week before death.

A summary of the data shows that this rabbit lost 2,105 cc. of blood and lived seventy-four days. During this time 55 Gm. of albumin and 93 Gm. of globulin were removed. The total loss in plasma protein was 148 Gm.

**Postmortem Examination:** Autopsy showed: great emaciation; tissues tough and leathery; no edema; no subcutaneous, mesenteric or perirenal fat; liver pale, without gross fatty metamorphosis; kidneys smooth

TABLE 3.—Rabbit 3

Date	Amount of Blood Taken, Cc.	Hemo- globin	Erythro- cytes	Leuko- cytes	Urine	Total Blood Volume, Cc.	Plasma Fats, Mg. per 100 Cc.			Total Protein, per Cent	Plasma Albu- min, per Cent	Plasma Globu- lin, per Cent	Non- Nitrogen, Mg. per 100 Cc.	Comment
							Saponi- fable	Nonsa- ponifiable	Total					
2/ 3/31	50	110	7,474,000	6,800	—	139	119	222	126	348	9.24	6.56	2.68	7½
2/ 7/31	50	..	.....	.....	—	...	...	310	91	401	6.40	3.39	3.11	36
2/ 7/31	50	..	.....	.....	—	...	...	334	80	414	6.58	3.38	3.20	36
2/ 9/31	50	..	.....	.....	Alb. +	...	...	436	95	531	5.43	4.38	1.05	30
2/11/31	50	58	3,100,000	5,000	Alb. +	182	124	488	129	617	4.82	3.08	1.74	41
2/14/31	50	..	.....	.....	Alb. +	...	...	512	111	623	4.82	2.89	3.98	32
2/16/31	50	..	.....	.....	Alb. +	...	...	540	80	620	7.49	2.76	5.73	30
2/18/31	50	40	1,984,000	9,600	—	155	110	319	82	401	6.48	2.76	4.70	32
2/22/31	60	50	.....	.....	Alb. +	...	...	344	89	433	6.80	2.73	4.07	29
2/24/31	55	50	2,976,000	6,600	—	...	...	387	91	478	6.93	2.54	4.39	34
2/26/31	60	45	.....	.....	Alb. +	...	...	535	84	519	6.25	1.83	4.42	34
2/28/31	60	40	.....	.....	Alb. +	...	...	597	93	690	9.14	2.01	6.53	35
3/ 2/31	45	35	2,010,000	10,600	—	119	102	757	99	856	8.61	1.78	6.83	47
3/ 4/31	45	28	.....	.....	Alb. +	...	...	1,251	169	1,420	9.25	2.00	7.25	21
3/ 6/31	45	25	.....	.....	Alb. +	...	...	1,359	201	1,560	8.88	1.61	7.27	34
3/ 9/31	55	35	.....	.....	—	...	...	911	102	1,031	7.25	2.26	4.99	46
3/11/31	45	45	2,720,000	7,600	Alb. +, casts	145	120	886	83	989	6.28	2.28	4.00	43
R. R. C., W. R. C.,														
3/14/31	50	50	.....	.....	Alb. +	...	...	937	89	1,026	6.75	2.23	4.52	44
3/16/31	60	35	3,440,000	27,800	Alb. +	...	...	1,193	98	1,291	6.44	2.25	4.19	45
3/18/31	55	30	.....	.....	—	...	...	779	150	929	6.52	2.93	3.59	49
3/21/31	50	35	.....	.....	—	157	113	678	169	847	6.41	...	...	43
3/23/31	60	30	.....	.....	—	...	...	1,499	291	1,790	6.78	2.60	4.18	60
3/25/31	60	35	.....	.....	Alb. +	...	...	1,519	301	1,820	8.29	...	...	30
3/27/31	45	30	3,500,000	15,200	Alb. +	...	...	1,755	288	2,043	7.25	3.30	4.95	33
3/29/31	60	32	.....	.....	—	...	...	1,944	356	2,300	7.93	2.81	5.11	45
3/30/31	60	28	.....	.....	—	...	...	2,736	204	2,940	7.68	2.59	5.10	43
4/ 1/31	60	25	2,400,000	12,600	Alb. +	128	102	2,667	214	2,881	...	...	...	...
4/ 2/31	50	20	.....	.....	—	...	...	2,792	271	3,063	7.67	2.13	5.54	24
4/ 4/31	65	28	.....	.....	—	...	...	2,922	174	3,096	8.23	2.50	5.73	20
4/ 5/31	60	25	.....	.....	—	...	...	1,622	162	1,784	7.62	2.29	5.23	30
4/ 7/31	60	30	.....	.....	—	...	...	2,084	502	2,586	7.96	2.93	6.03	41
4/ 8/31	60	28	.....	.....	—	...	...	3,401	273	3,674	8.37	2.14	6.23	20
4/10/31	60	25	2,100,000	8,600	—	...	...	.....	97	1,095	6.63	2.00	4.63	40
4/12/31	60	20	.....	.....	—	104	87	998	...	...	6.80	2.08	4.73	36
4/14/31	60	30	.....	.....	—	...	...	.....	...	3,355	7.01	2.75	4.26	53
4/17/31	70	30	.....	.....	—	...	...	4,549	301	4,850	6.98	2.81	4.17	40
4/18/31	70	30	2,296,000	12,700	—	...	...	1,963	297	2,260	6.70	2.01	4.69	31
								2,193	271	2,464	6.07	2.21	4.86	41

Listless, poor appetite  
Listless, poor appetite  
Listless, poor appetite  
Listless, poor appetite

Very listless

Emaciated, watery  
diarrhea  
Emaciated, watery  
diarrhea  
Emaciated, watery  
diarrhea  
Emaciated, watery  
diarrhea

and pale; renal cortex and medulla both pale; remaining tissues very pale; practically no blood in heart and large vessels; bone marrow very red throughout.

**Microscopic Examination:** Sections of the kidneys stained with hematoxylin-eosin were normal. Fresh frozen sections stained with sudan III failed to show fat of any kind. Sections of the liver stained with hematoxylin-eosin showed some destruction of the cords. Sections stained with sudan III did not show fat.

*Rabbit 4* (table 4).—A severe anemia developed rapidly, and after the second bleeding the plasma was distinctly milky. There was a true increase in plasma fat, which persisted throughout the lifetime of the animal. This increase was most pronounced in the neutral fat fraction. The milky appearance of the plasma was lost about four days before the rabbit died; nevertheless the amount of extractable fat remained greatly increased.

A slight edema of the scrotum was noted on the ninth day after bleeding was started. The plasma lipoids at the time were 1,603 mg. per hundred cubic centimeters; the total plasma protein was 6.37 per cent; the albumin, 2.53 per cent, and the globulin, 3.84 per cent. There was no striking deviation from these values throughout the remainder of the experiment. On the last analysis, the albumin fell to 1.88 per cent. The edema rapidly increased and soon involved the greater part of the anterior abdominal wall. Massive ascites was demonstrable on the twentieth day, and the animal now had difficulty in walking.

After the twelfth day, the plasma nonprotein nitrogen began to increase and on the day of death rose to 144 mg. per hundred cubic centimeters. The blood and plasma volumes fell as the bleeding progressed.

The urine began to show large quantities of fat soon after the lipemia was well established. This was chiefly neutral fat. The urine was albumin-free for the most part. Three of the analyses showed a small amount.

In spite of the marked subcutaneous edema and ascites, the rabbit lost weight and became emaciated. A watery diarrhea developed a few days before the animal's death.

A summary of the data shows that the rabbit survived twenty-four days with a blood loss of 615 cc. Eighteen grams of albumin and 21 of globulin were removed. The total amount of plasma protein lost was 39 Gm.

**Postmortem Examination:** The observations were: great emaciation; marked decrease in subcutaneous, mesenteric and perirenal fat; scrotum four times normal size; subcutaneous tissue of scrotum and anterior abdominal wall glassy, gelatinous and about 2 cm. thick; no hydrocele; about 30 cc. of clear, straw-colored fluid in peritoneal cavity;

TABLE 4.—*Rabbit 4*

Date	Amount of Blood Taken, Ce.	Hemo- globin	Erythro- cytes	Leuko- cytes	Urine	Total Blood Volume, Ce.	Plasma Ce.	Plasma Fats, Mg. per 100 Ce.			Total Plasma Protein, per Cent per Cent 100 Ce.	Plasma Albu- min, mg. per Cent 100 Ce.	Plasma protein Glob. Nitrogen, Mg. per Cent 100 Ce.	Comment
								Saponi- fiable	Nonsa- ponifiable	Total				
3/ 9/31	50	105	7,040,000	7,200	—	164	100	702	72	772	8.17	4.93	41	6½
3/12/31	40	60	.....	.....	—	...	...	542	16	558	7.14	5.32	45	6½
3/14/21	50	55	.....	.....	—	...	...	876	106	1,082	6.00	2.83	54	6
3/16/31	45	45	.....	.....	—	90	65	1,156	56	1,212	7.01	2.34	47	6
3/18/31	55	30	.....	.....	—	...	...	1,502	102	1,603	6.37	2.53	45	5¼
3/21/31	50	25	800,000	14,000	—	...	...	1,808	674	2,572	7.21	2.34	45	5½
3/23/31	60	25	.....	.....	—	...	...	919	183	1,102	6.06	3.48	60	5½
3/25/31	60	25	.....	.....	Alb. +	64	53	1,458	141	1,602	5.37	2.00	76	5½
3/27/31	45	25	1,520,000	10,000	—	...	...	1,142	64	1,206	5.35	2.57	80	5¼
3/29/31	60	22	.....	.....	Alb. +	...	...	1,425	126	1,551	5.77	2.39	89	5¼
3/31/31	45	20	1,840,000	13,000	—	...	...	508	736	1,244	5.81	2.81	95	5
4/ 2/31	55	15	1,120,000	12,000	Alb. +	...	...	1,309	235	1,544	6.36	1.88	144	5

Slight edema of serotum  
Increasing edema of serotum  
Edema of serotum  
Edema of serotum  
Edema of serotum  
Edema of serotum and anterior abdominal wall  
Asciates  
Edema of serotum and anterior abdominal wall  
+++ , asciates, watery diarrhea

bilateral hydrothorax; marked fatty metamorphosis of the liver; some fat in spleen; kidneys smooth and pale; renal surfaces golden yellow; renal cortices golden yellow; renal medullae very pale and all markings lost; bone marrow yellow except at extreme ends.

**Microscopic Examination:** Sections of kidneys stained with hematoxylin-eosin showed a moderate destruction of the convoluted tubules with some round cell infiltration. There was a slight increase in interstitial tissue. The glomeruli were normal. Fresh frozen sections stained with sudan III showed some overstaining of the cytoplasm of the convoluted tubules. There were no fat droplets. Sections of the liver stained with hematoxylin-eosin showed moderate vacuolization of the cytoplasm of the cords. Fresh frozen sections stained with sudan III showed a moderate amount of fat.

#### SUMMARY OF RESULTS

Of the original fifteen rabbits, only four lived long enough to be of value. All were subjected to exactly the same procedure. In two of the animals (nos. 1 and 4) there developed edema of the scrotum and anterior abdominal wall, massive ascites and hydrothorax. Rabbit 1 also had moderate edema of the hind legs. The edema rapidly subsided if the bleeding was discontinued (rabbit 1), but soon returned when the bleeding was resumed. The remaining two never showed a trace of edema.

All the rabbits were bled until they died. The average duration of life from the beginning of the bleeding was about one month. Rabbit 3 was unusual in that it survived nearly two and one-half months. The total amount of blood removed ranged from 615 cc. (rabbit 4) to 2,105 cc. (rabbit 3). The amount of plasma protein actually lost ranged from 39 to 148 Gm. This is considerably more than is indicated by the protein concentrations expressed in percentages. All this is, however, consistent when the great decrease in blood volume and plasma volume is considered.

After several bleedings, all the animals presented a lipemia. The time of onset of the lipemia varied considerably in the different rabbits. Lipemia secondary to repeated hemorrhage in rabbits was first reported by Boggs and Morris<sup>9</sup> in 1909. Since then lipemia in rabbits has been similarly produced by Milne,<sup>10</sup> Sakai,<sup>11</sup> Horiuchi,<sup>12</sup> Fishberg and Fish-

9. Boggs, T. R., and Morris, R. S.: *J. Exper. Med.* **11**:553, 1909.

10. Milne, L. S.: *Deutsches Arch. f. klin. Med.* **109**:401, 1912-1913.

11. Sakai, S.: *Biochem. Ztschr.* **62**:387, 1914.

12. Horiuchi, Y.: *J. Biol. Chem.* **44**:345, 1920.

berg,<sup>13</sup> Fishberg,<sup>14</sup> Heki<sup>15</sup> and Johansen.<sup>16</sup> None of these workers were ever able to produce edema in their rabbits. Fishberg and Fishberg suggested that there might be some retention of water in their animals even though edema was never demonstrable. As the bleeding was continued, the lipemia became more marked, but at no time did it reach or maintain a constant level. On the whole, the plasma lipoid level was very irregular throughout. A milky plasma did not always indicate a high lipoid content and vice versa. In all cases, the increase was primarily in the neutral fat (saponifiable) fraction. Sakai, Horiuchi and Fishberg and Fishberg made the same observation. The sterol (nonsaponifiable) fraction showed only a moderate increase as a rule. Occasionally, however, there was a sudden marked rise.

The total plasma protein was slightly decreased as the bleeding progressed. The lowest value encountered was 4.51 per cent (rabbit 1), but this was only transitory and rapidly increased to 7.67 per cent. In the other three animals, the total plasma protein never fell below 5 per cent. Occasionally it rose to 8 and 9 per cent.

In all cases, the albumin showed a constant and fairly uniform decrease. There was a simultaneous increase in the globulin. The albumin-globulin ratio was reversed for the most part. In rabbits 1 and 4, in which edema developed, the final values for albumin fell to 1.87 and 1.88 per cent, respectively. The edema, however, came on when the albumin was approximately 2.5 per cent, and in rabbit 4 even rose to over 3 per cent just when the edema was becoming marked. Similar figures for albumin (2.5 per cent) in rabbits 2 and 3 were constantly found, yet these animals never showed edema. The lowest percentage of albumin (1.61 per cent) was found in a nonedematous rabbit (no. 3).

The total nonprotein nitrogen showed a terminal increase in the edematous rabbits (1 and 4). It remained within normal limits in the nonedematous animals (2 and 3).

A marked secondary anemia developed in all the animals, although this came on quicker and became more pronounced in some animals than in others.

All four rabbits lost weight and became greatly emaciated as the bleeding progressed. This was especially noticeable in the nonedematous ones. They became listless, and usually began to have a watery diarrhea a few days before death.

The urine at times showed a small amount of albumin after the onset of the lipemia. This was seldom more than a trace and was

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13. Fishberg, E. H., and Fishberg, A.M.: *Proc. Soc. Exper. Biol. & Med.* **25**:296, 1927.

14. Fishberg, E. H.: *J. Biol. Chem.* **81**:205, 1929.

15. Heki, M.: *J. Biochem.* **11**:369, 1930.

16. Johansen, A. H.: *J. Biol. Chem.* **88**:669, 1930.

always inconstant. Rabbits 1 and 3 on one occasion showed a few white blood cells, red blood cells and granular casts. Fat appeared in the urine as the lipemia progressed. It showed about the same values for saponifiable and nonsaponifiable fat as did the fat in the plasma.

At autopsy all animals were greatly emaciated. The subcutaneous mesenteric and perirenal fat was almost gone. Boggs and Morris, Sakai, Horiuchi, Fishberg and Fishberg and Johansen found the same condition in their rabbits. The viscera were pale and anemic. In rabbits 1, 2 and 4 there was a well marked fatty metamorphosis of the liver, the fat being most prominent at the periphery of the lobules. The surfaces of the kidneys were smooth and golden yellow. On section, the medullae were very pale, while the cortices, especially the peripheral parts, showed the same golden yellow color as the surface. The bone marrow, with a single exception (rabbit 3), was red only at the ends of the long bones.

In addition to the aforementioned findings, rabbits 1 and 4 showed

TABLE 5.—*Analyses of Edema Fluids*

Rabbit	Total Protein, per Cent	Albumin, per Cent	Globulin, per Cent	Nonprotein Nitrogen, Mg. per 100 Cc.	Source of Fluid
1	5.77	1.73	4.04	125	Ascitic fluid
	2.44	1.10	1.34	81	Subcutaneous fluid
4	3.51	1.57	1.94	146	Ascitic fluid
	3.65	1.06	2.59	72	Subcutaneous fluid

a massive edema of the scrotum, massive ascites and hydrothorax. There was also a moderate subcutaneous edema of the hind legs in rabbit 1. The subcutaneous tissue measured from 0.5 to 1.5 cm. in thickness, and displayed a glassy gelatinous appearance. A clear, watery fluid could be easily expressed by simply squeezing the tissue.

Analysis of the subcutaneous fluid showed it to be fairly high in protein (table 5). The protein content of the ascitic fluid was likewise high (table 5). In all the edema fluids, the globulin was in excess of the albumin, and in general reflected the protein changes in the blood plasma.

Rabbit 3 (nonedematous) was bled over a period of nearly two and one-half months with a total blood loss of 2,105 cc. Unlike the other three animals, this one showed no gross evidences of fatty changes in the liver. Subsequent analysis of the liver showed the fat content to be entirely within normal limits (table 6). The bone marrow was red throughout the shafts of the long bones.

The kidneys of all four rabbits were analyzed for water and lipid content (table 7). In addition, the livers of rabbits 3 (nonedematous) and 4 (edematous) were similarly analyzed (table 7). For comparison, two normal rabbits were included.



The kidneys of the edematous (1 and 4) rabbits showed a slight increase in water and a rather marked increase in lipoids. Conversely, the kidneys of the nonedematous animals (2 and 3) showed a normal water content, and the total lipoids, although slightly higher than the normal, were lower than those found in the edematous animals. The striking difference was in the saponifiable and nonsaponifiable fractions. In the edematous group, the nonsaponifiable fraction fell from the normal of from 63 to 70 per cent to 1 and 5 per cent. There was a great increase in neutral fat. One of the nonedematous rabbits (2)

TABLE 6.—*Analyses of Fats in Liver*

Rabbit	Fresh Liver, Gm.	Dried Liver, Gm.	Water, per Cent	Fat,* per Cent	Saponifiable Fat, per Cent	Nonsaponifiable Fat, per Cent
Normal	9.09	2.84	69	14	94	6
4†	10.57	2.56	76	36	97	3
3	12.85	3.14	75	19	92	8

\* Calculated on the dry weight.

† Edematous.

TABLE 7.—*Analyses of Fats in Kidney*

Rabbit	Fresh Liver, Gm.	Dried Liver, Gm.	Water, per Cent	Fat,* per Cent	Saponifiable Fat, per Cent	Nonsaponifiable Fat, per Cent
Normal	17.62	3.89	78	13.6	37	63
Normal	4.98	1.81	77	24.0	30	70
1†	12.81	2.23	83	38.0	99	1
4†	11.25	2.17	81	37.0	95	5
2	5.93	1.40	76	25.0	40	60
3	4.38	0.93	79	30.0	81	19

\* Calculated on the dry weight.

† Edematous.

showed practically normal values for all the lipid fractions. In rabbit 3 (nonedematous), however, the nonsaponifiable fractions fell to 19 per cent.

Identical analyses were made on the liver of one edematous (4) and one nonedematous (3) rabbit. Both showed a slight increase in water, but only the edematous animal had an increase in total lipid. There was nothing significant in the saponifiable and nonsaponifiable fractions.

Hematoxylin-eosin sections of the kidneys of rabbits 1, 2 and 3 were essentially normal. The kidneys of rabbit 4 showed some tubular destruction (especially of the convoluted tubules) and a moderate round cell infiltration into the interstitial tissue. In certain places there was a slight increase in the interstitial fibrous tissue. The glomeruli appeared normal. This was clearly a case of spontaneous nephritis in the rabbit.

Fresh frozen sections of the kidneys of all animals stained with sudan III showed the cytoplasm of the convoluted tubules somewhat overstained with sudan III, but no distinct fat droplets were ever

demonstrable. These sections were compared with sections of normal kidneys subjected to the same stain in all cases.

Sections of the liver stained with hematoxylin-eosin and fresh frozen sections stained with sudan III all showed a marked increase in fat.

#### COMMENT

There was a marked difference in the reaction of the different rabbits. In general, however, they fell into two groups: the one in which a moderate amount of bleeding was soon followed by a marked edema; the other in which even prolonged bleeding failed to produce edema. The anemia was equally marked in each case. The animals in which edema developed showed a sharp rise in plasma lipid just when the edema became demonstrable, and the lipid continued to be elevated while the edema was present. Discontinuance of the bleeding caused both the lipemia and the edema to disappear (rabbit 1). Rabbit 2 (nonedematous) showed low plasma lipid values for the most part, but at times there would be a sudden rise just as marked as in the edematous animals. In the other nonedematous rabbit (3) lipemia was very slow in developing, but once established it became the most marked of all. Changes in the total concentrations of total plasma protein, albumin and globulin were practically the same in both the edematous and the nonedematous rabbits. The actual amount of total plasma protein removed ranged from 39 to 148 Gm. Both the total amount of plasma protein removed and the resulting plasma protein concentration must, of course, be interpreted in the light of the decreased blood volume. Edema became demonstrable while the concentration of total plasma protein was still within normal limits, and while the concentration of albumin was around 2.5 per cent. It is true that the edematous animals showed terminal albumin concentrations of 1.87 per cent and 1.88 per cent, but the edema had been present for days before this. An inverse relationship between the concentrations of total plasma protein and albumin and those of plasma lipids as described by Fishberg was generally present. Blood volume was decreased to about the same degree in both the edematous and the nonedematous animals. It would appear, therefore, that the development of edema in rabbits is not directly related to the concentration of total plasma protein or to that of albumin or to that of lipids. The osmotic pressure of the plasma under such conditions might offer a clue.

The possibility of the repeated heart punctures causing the edema is ruled out by the fact that edema never developed in two of the animals, even though in one of these (rabbit 3) the heart was punctured twice as often as in any of the rest.

The edema fluids of the rabbits were exceptionally high in protein (table 5). In this they were in direct contrast with the edema fluids of dogs in which the edema was produced by plasmapheresis. When bleeding alone was used to induce the edema, there was an actual depletion of water in the organism. Oddly, the animals did not manifest an increased thirst under such conditions. The rabbits used derived most of their water from the fresh vegetables that were fed them and drank little. In the edema of dogs produced by plasmapheresis, water was continuously added in the form of Ringer's or Locke's solution. Large amounts of water were also given by stomach tube by some of the workers. This added amount of water may explain a part of the difference in the protein concentration in the edema fluids.

It has already been suggested that it is difficult to explain the development of edema in a rabbit that is suffering a continuous loss of water on the observed alterations in the concentrations of the plasma proteins and lipoids. It must be assumed that there is some increased affinity or "thirst" of the tissue colloids for water. Without this assumption it is difficult to understand how the tissues could draw water from the blood and hold it when the blood volume is depleted and no extra fluid is added in any way.

From the comparative analyses of the liver and kidneys of normal rabbits and those with a marked and prolonged lipemia it would seem that the excess fat in the kidneys and in the liver is probably derived from the blood. The increase here, as in the blood plasma, is primarily in the neutral fat fraction.

The reduction in the nonsaponifiable (sterol) fraction with the simultaneous increase in the neutral fat portion in the kidneys of the edematous rabbits is striking (table 6). A satisfactory explanation of this shift is difficult to offer. It is practically impossible to assume from the known chemical structures of the fatty acids, neutral fats and sterols that there could be a conversion from a sterol to a neutral fat. From the given experimental data it is impossible to say whether this bears any relation to the development of the edema.

It was interesting to note that while fresh frozen sections of the kidney stained for fat failed to show stainable fat, the chemical analysis demonstrated a marked increase. The fat in the liver was always stainable.

It is probable that the excess fat in the plasma was derived from the fat depots of the body. The fact that the increase in plasma lipid was primarily in the neutral fat fraction gives support to this theory. In all cases of marked lipemia, the rabbits at autopsy showed a striking decrease in subcutaneous, mesenteric and perirenal fat. At times there was practically no demonstrable fat. Similar observations were reported by Boggs and Morris, Sakai, Horiuchi, Fishberg and Fishberg and Johansen.

A satisfactory explanation for the accumulation of fat in the blood has never been offered. Boggs and Morris suggested that the lipemia was due to defective oxidation secondary to the anemia. Griffel<sup>17</sup> was able to induce a lipemia in rabbits by letting them live for a time in rarefied air. Sakai found a decrease in blood lipase and suggested this as the cause for the excess fat in the blood. Fishberg thought it was secondary to the loss of plasma protein.

It is interesting to note in this connection that it is very difficult, if not impossible, to induce lipemia in rabbits when phenylhydrazine (Heki) or pyrodine (Boggs and Morris) is used to bring on the anemia.

Some of the excess plasma fat is excreted as such by the kidney and can be recovered in the urine. This excretion of fat by the kidney goes on for the most part without any demonstrable histologic changes in the kidney. It is true that the kidneys of edematous rabbit 4 showed some tubular destruction with some round cell infiltration, but this obviously was a spontaneous nephritis in the rabbit, a disease to which rabbits are prone. There is no direct evidence that it was secondary to any experimental procedure.

A long-standing edema cannot be produced in the rabbit by simple bleeding, because the animal always dies as the bleeding is continued. On the other hand, if the bleeding is discontinued after the edema has become marked, the edema at once subsides and the rabbit recovers.

*Relationship of Experimental Edema and Lipemia to Lipoid Nephrosis.*—There are some points of resemblance between this experimental disease of rabbits and human lipoid nephrosis. Both show edema, lipemia and a decrease in plasma albumin.

In the lipemia of rabbits, the increase in plasma fat is primarily in the neutral fat fraction. In lipoid nephrosis, the increase in plasma lipid is likewise in the neutral fat portion (unpublished work).

In lipoid nephrosis, the patient loses plasma protein, especially albumin, through damaged glomerular capillaries. In the experimental work here reported, plasma protein was removed by bleeding. In both cases, the albumin is greatly reduced, while the globulin is markedly increased in the rabbit and normal or slightly increased in man. The rabbits in this series did not show as great a reduction in the percentage of total protein as is generally encountered in lipoid nephrosis. However, if one takes into consideration, the decrease in blood volume, there is a marked decrease in total plasma protein even though the concentration at the time failed to show a striking decrease. The albumin and globulin concentrations must be similarly interpreted.

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17. Griffel, W.: *Biochem. Ztschr.* **222**:290, 1930.

Whether or not there is any etiologic relationship between the experimentally produced edema in rabbits and the edema of lipid nephrosis must remain an open question for the time being. The experimental work suggests that the lipemia and perhaps the edema are caused in some way by the loss of plasma protein in both cases. The resulting concentrations of plasma protein and plasma albumin in the edematous rabbit are much higher than in the patient with lipid nephrosis, even though there is a decrease in the actual amounts of the total protein and albumin. It is difficult, therefore, to explain the sudden onset of the edema in rabbits on the basis of an altered concentration of plasma protein or of albumin. An increased affinity for water on the part of the tissue colloids would help to explain the transfer of water and the resulting edema. How a loss of plasma proteins brings about lipemia and edema remains to be determined.

The urine of the rabbits was always high in fat. The protein content of the edema fluids of the rabbits (table 5), especially the subcutaneous fluid, was considerably higher than that usually described as typical of lipid nephrosis. It was likewise higher than the protein content of the edema fluids in the dogs described by Leiter and by Shelburne and Egloff, in which edema was produced by plasmapheresis.

The histologic structure of the kidneys in these experimental animals was entirely different from that of the kidneys in human lipid nephrosis.

#### CONCLUSIONS

Edema was produced in two of four rabbits by prolonged bleeding alone.

All rabbits showed a marked and persistent lipemia. The increase in plasma lipid was primarily in the neutral fat fraction, the sterol fraction as a rule being less increased.

Total blood volume and plasma volume fell as the bleeding progressed. This occurred in both the edematous and the nonedematous animals, and was about the same in each group. The concentration of total plasma protein fell but slightly; that of the albumin was moderately lowered and that of the globulin at times greatly increased. Reversals of the albumin-globulin ratio were frequently encountered.

The edematous rabbits showed a marked edema of the scrotum and anterior abdominal wall, massive ascites and hydrothorax. All edema fluids were high in protein.

The urines of all the animals, both edematous and nonedematous, were high in fat. This was mostly neutral fat. There was a variable increase in the lipid content of the kidney and liver in all instances. In the kidneys of the edematous rabbits there was a striking decrease in sterols, with an equally marked increase in neutral fat.

# Laboratory Methods and Technical Notes

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## A RAPID METHOD FOR STAINING FROZEN SECTIONS \*

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This method of staining combines the use of a rapid and permanent nuclear stain and that of a counterstain. It has been found extremely valuable in the operating room and in routine diagnostic study of frozen tissues.

### TECHNIC

*Apparatus and Reagents.*—Apparatus and materials to be used are: Spencer freezing microtome and accessories, microscope, five dropping bottles (capacity 30 cc.), two petri dishes, one porcelain dish, tripod, bunsen burner, one glass rod teaser, fine blotter, coverslips, glass slides, 80 per cent alcohol, 95 per cent alcohol, clearing and dehydrating solution, iron hematoxylin, alcoholic solution of eosin, Canada balsam and 10 per cent formaldehyde.

*Preparation of Stains and Clearing Solution.*—The iron hematoxylin solution is a modification of Weigert's iron hematoxylin. It is prepared as follows: Stock solutions A and B are first prepared.

Stock Solution A: Hematoxylin (Grübler), 1 Gm.; 95 per cent alcohol, 100 cc.

Stock Solution B: Ten per cent ferric chloride (Merck), 5 cc.; glacial acetic acid, 15 cc.; distilled water, 80 cc.

Both stock solutions are to be kept in a dark place. It is preferable that solution A be allowed to age for from two to three weeks before using. It should be discarded after six months.

The nuclear stain is prepared by mixing well 2 parts of solution A with 1 part of solution B. It is allowed to stand about one hour before using. The indicated proportions must be carefully measured, as a slight variation in the concentrations of the acid and ferric chloride tends to render the stain less stable. When solutions A and B are mixed, an intense blue color develops that gradually assumes a deep purple, and finally a deep red-brown, color. If carefully prepared, this solution is ready to use within an hour and retains its staining properties well for from three to four weeks. If a green color develops in the stain, it is unfit for use. This color is due either to the use of unclean glassware or to too great concentrations of acetic acid and ferric chloride. It is preferable that the stain be kept in a dark amber bottle with a ground glass stopper.

The counterstain is prepared as follows: Dissolve 2.5 Gm. of water-soluble yellow eosin in about 10 cc. of distilled water and make up to 500 cc. with distilled water. Add 4 cc. of concentrated hydrochloric acid, drop by drop. A precipitate forms, which settles to the bottom. Pour off the supernatant fluid and wash six

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\* Submitted for publication, Sept. 17, 1931.

\* From the laboratories of the Lois Grunow Memorial, Phoenix, Ariz., and the Department of Pathology, University of Minnesota, Minneapolis.

times with distilled water. Then filter and allow to dry in the oven at about 100 F. for twenty-four hours. Scrape off the residue from the filter paper and place it in the bottle. To each 0.5 Gm. add 100 cc. of 80 per cent alcohol. This solution keeps permanently.

The dehydrating-clearing solution is prepared after the method of Kumagai<sup>1</sup> and consists of the following reagents:

	Cc.
Xylene .....	7.5
Phenol .....	2.5
Absolute alcohol .....	10.0

*Staining.*—Very thin sections of tissue are fixed in 10 per cent formaldehyde for twelve hours, if time permits, or for three minutes in boiling 10 per cent formaldehyde, if a rapid diagnosis is required. The technic of sectioning frozen tissue described by Broders<sup>2</sup> has been found very satisfactory. It is recommended that the reader refer to this article, since many points are brought out that are of importance in the technic of obtaining frozen sections. The rapidity of this method rests almost entirely on the efficiency and speed of obtaining sections, as the staining procedure requires less than one minute. With proper technic, sections may be obtained that are not greater than 10 microns in thickness. Thick sections are not satisfactory, as proper dehydration and clearing are not easily obtained.

The sections are placed in shallow petri dishes containing water. A thin section is selected and floated on the surface of a clean glass slide. The section is then spread out by dropping 80 per cent alcohol directly over its surface. In this manner, practically all of the wrinkles in the section may be removed. The excess alcohol is drained off, and a blotter is placed firmly over the section, with the use of a moderate amount of pressure to avoid displacement. In this manner, it has been found that the section adheres closely to the slide and is not removed by blotting, whereas if the section is blotted without first using alcohol, it very often adheres to the blotter. Care must be taken to allow as little time as possible to elapse between blotting and staining, so that drying of the tissue is prevented.

The following procedure is employed in staining the section:

1. Cover the section with iron hematoxylin and allow it to stain for from five to ten seconds.
2. Wash off with 80 per cent alcohol.
3. Cover the section with eosin.
4. Wash immediately with 80 per cent alcohol.
5. Wash quickly with 95 per cent alcohol.
6. Treat with a few drops of dehydrating and clearing solution.
7. Mount in balsam.

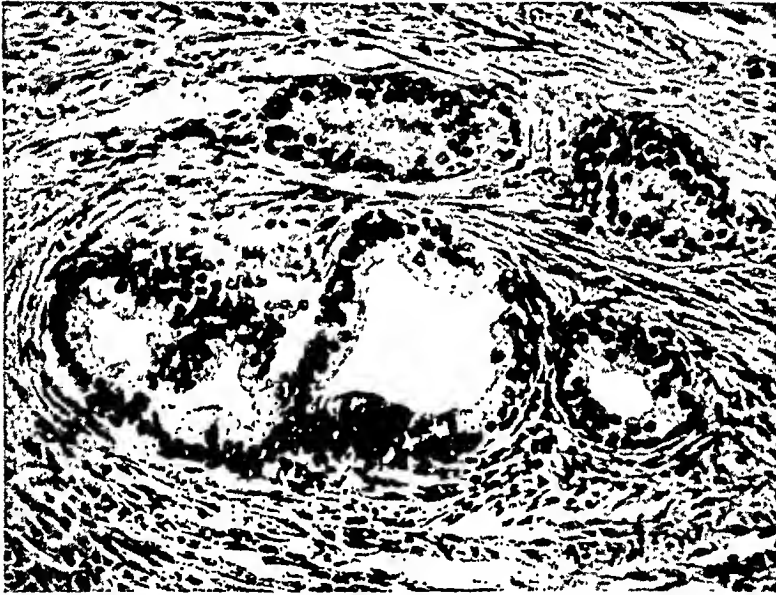
The stain and other fluids are dropped directly on the section. In dehydrating and clearing, the slide is held at an angle, and the reagents are allowed to drain into a petri dish. From 10 to 15 drops of the alcohols and dehydrating and clearing reagents are sufficient. From 4 to 5 drops of the stains are ample for the section of average size.

1. Kumagai, Kuranosuke: *Folia anat. japon.* **3**:31, 1925.

2. Broders, A. C.: *J. Lab. & Clin. Med.* **16**:734, 1931.

## COMMENT

By using proper concentrations of iron hematoxylin and eosin, and a short exposure of the tissues to the stain, it has been found that differentiation, other than that obtained by the use of the alcohols and the dehydrating and clearing solutions, is not necessary. The time required for this stain is therefore much shortened. Clear nuclear staining may be obtained in tissues that are cut thin and properly fixed. The accompanying photomicrograph shows the fine detail of cytoplasm and nucleus obtainable by this method. The nucleus is of a deep blue color with stains that are from one hour to one week old. With



Hyperplasia of prostate;  $\times 80$ ; frozen section, stained with iron hematoxylin and eosin.

older stains, the nucleus is of a blacker color. The cytoplasm is clearly defined and purplish red.

If sections are thin, proper clearing is easily accomplished by this method, and the sections retain their color permanently. If sections are thick, clearing may be continued after examination of the slide by removing the balsam and allowing the sections to remain in the xylene for several minutes.

The use of Kumagai's clearing and dehydrating reagent has been found very satisfactory, as proper clearing is accomplished without previous dehydration with absolute alcohol. Also much of the shrinkage that is produced by the use of xylene alone is avoided.



# General Reviews

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## SYPHILITIC MYOCARDITIS \*

O. SAPHIR, M.D.

CHICAGO

*(Concluded from page 295)*

### HISTOLOGIC CRITERIA OF SYPHILITIC MYOCARDITIS

In reviewing the literature casually one gains the impression that syphilitic myocarditis is by no means rare. Seemingly many cases are reported, and one may therefore be tempted to agree with Warthin, who stated that old latent syphilis is one of the most important causes, if not the most important cause, leading to myocardial incompetency. A closer and more critical inspection of the literature on this subject, however, reveals that in many cases either the diagnosis "syphilis" or the diagnosis "myocarditis" was obviously not based on histologic facts. Changes in the myocardium found incidentally in cases of syphilis in other organs are often interpreted as syphilitic in origin because of the evidence of syphilis elsewhere. On the other hand, fibroses of the myocardium as the result of vascular disturbances are often thought to be remnants of myocarditis.

In the following paragraphs I shall try to give the histologic criteria of syphilitic myocarditis in cases reported in the literature.

Virchow stated that one finds in the heart in cases of syphilis multiple fibrous scars without any other demonstrable cause but syphilis. He mentioned, however, that it is difficult to prove that such lesions are syphilitic. Mueller believed that four of his reported cases showed syphilitic myocarditis, because there was no other etiology for the myocarditis found at autopsy, because the same type of fibrosis is also found in hearts that show gummas, and because of the similarity of the changes in the myocardium in his cases and the changes found in syphilitic orchitis and interstitial syphilitic hepatitis. Ehrlich, probably the first to describe endarteritic changes of the coronary vessels, believed that the fibrous myocarditis in the case that he reported was the result of those vascular changes. Histologically, the myocardium showed necrosis, a marked cellular infiltration, many capillaries and a more or less prominent invasion of pus cells. The myocarditis in

Chvostec's case was thought to be of syphilitic origin because of the coincidental syphilitic lesions in the brain. Mracek believed that the combination of old and newly formed connective tissue with granulation tissue is characteristic of syphilitic myocarditis. He also stated that the diagnosis of syphilis can be made only by the analogy of the changes to syphilitic changes in other organs. Loomis said that syphilis gives rise to an indurated myocarditis, a condition that in its later stages is hardly distinguishable from fibrous disease due to other causes. It has been possible to infer the origin of this fibrosis only from the antecedent history of the patient. Stolper found in his cases a cellular infiltration of the adventitia of the smaller branches of the coronary arteries and endarteritis obliterans. Herrick stated that the fibrous form of myocarditis is characterized by grayish areas or streaks in the myocardium. These areas when fully fibrous differ in no respect from fibrous myocardial patches due to coronary obstruction with consequent myomalacia and subsequent scarring. He mentioned that the diagnosis of syphilitic myocarditis must be based on probability. Adler's case showed a gumma in the suprarenal gland; the heart was the seat of a cellular infiltration of the myocardium with some coagulation necrosis, but no vascular changes. Wagner and Qwiatkowski based their diagnosis of syphilitic myocarditis on the finding of much fibrous tissue in the midst of the muscle tissue, with many spindle-shaped cells, necrotic portions and endarteritis of branches of the coronary arteries. Herxheimer stressed the point that an infiltration of round cells of the interstitial tissue along the blood vessels is characteristic of syphilitic fibrous myocarditis; scar tissue, mainly in the left ventricle close to the apex, is also often encountered. Landois found much connective tissue in the myocardium with only a few nuclei. There were cells surrounding small blood vessels, the lumina of which were obliterated; the larger vessels showed no changes. Takata's case revealed circumscribed perivascular infiltrations, mainly of round cells and plasma cells; there was a new formation of connective tissue present with some polymorphonuclear leukocytes. Some of the smaller arterioles and venules showed a thickened intima. He believed that circumscribed infiltrations mainly of plasma cells about the venules are characteristic of syphilitic myocarditis. Spalding and von Glahn based their diagnosis on the finding of necrosis and polymorphonuclear leukocytes and on the presence of a moderate number of spirochetes. Hines found recent and old infarcts in the heart; histologically, granulation tissue with polymorphonuclear leukocytes, a perivascular infiltration by lymphocytes and some fibrous scarring were present. He also mentioned finding spirochetes. Boyd found lymphocytes and plasma cells in the adventitia of the coronary artery, and in addition polymorpho-

nuclear leukocytes. He also claimed to have found spirochetes. Wilson reported patches of sclerosis and an infiltration by round cells in the interstitial tissue of the myocardium; spirochetes were found in the interventricular septum. Gravier found much interstitial connective tissue with plasmacytes and edema. Many capillaries were found in some portions and much fibrosis in other fields. Chaniotis found a diffuse fibrous myocarditis consisting histologically of connective tissue, degenerative changes of the muscle fibers and edema.

The criteria of syphilitic involvement of the myocardium as given by Warthin will now be referred to in detail.

In 1914 he described the cardiac lesions produced by *Spirochaeta pallida*. He stated that it had long been suspected that syphilis played an important rôle in the production of cardiac disease. The truth of the matter was, he said, that before the discovery of *Spirochaeta pallida* as the etiologic agent of syphilis, pathologists recognized but few lesions of the heart as essentially pathognomonic of syphilis. As to the significance of the gumma, there were no doubts, but gumma of the heart was relatively rare, and actual proof of cardiac syphilis was restricted to the instances in which the gumma was present. The frequent association of forms of chronic myocarditis, fibrous heart, anemic infarction of the myocardium and coronary sclerosis, with other pathologic evidences of syphilis elsewhere in the body (such as tabes, dementia paralytica, gumma of the brain or of the liver, aortic aneurysm, lesions of the bones, orchitis fibrosa, etc.) had given pathologists a strong leaning toward the view that the heart was one of the organs most frequently affected in syphilis. That these lesions were actually syphilitic could be assumed only on the strength of circumstantial evidence before the demonstration of *Spirochaeta pallida*. Warthin claimed to have found large colonies of spirochetes in the myocardium, either in the tissue spaces of certain muscle areas or about the blood vessels, without any changes in the adjacent cardiac muscle that could be recognized by any of the technical methods employed at the present time. Studies of cardiac syphilis showed, he said, that the primary lesions produced by the spirochetes may be either parenchymatous or interstitial. The parenchymatous lesions are a peculiar pale degeneration, fatty degeneration, simple atrophy and necrosis; the interstitial lesions are a peculiar form of edema (myxedema), vascular and perivascular infiltrations and localized myxoma-like formations. The parenchymatous changes may occur absolutely independently of the interstitial, and the latter may be found with no associated changes in the adjacent cardiac muscle. The more marked the interstitial changes, the more likely are parenchymatous changes to be associated with them, but the most marked parenchymatous lesions may occur without any interstitial changes. The purely parenchymatous lesions

are found especially in virulent congenital, active secondary and early tertiary syphilis. In milder and older infections the interstitial changes, particularly the localized vascular and perivascular proliferations, predominate. The myxoma-like formations resembling undifferentiated gummas also occur in more localized and milder infections. It is also of great importance to know that the heart is so frequently the seat of localization of spirochetes. The cardiac localization of spirochetes is more common than the hepatic. Spirochetes may be found in great numbers in the heart when they can be found nowhere else in the body. In such cases the cardiac muscle may also show no lesions that, according to older knowledge, would be classed as syphilitic; indeed, no organ or tissue may show any histologic sign of syphilis even when the organisms are present in great numbers.

In 1918, in his "New Pathology of Syphilis," Warthin described the myocardium in syphilis. To the naked eye, the hearts show, as a rule, dilatation, hypertrophy, atrophy and fibroid patches in the wall of the left ventricle. The determination of cardiac syphilis is essentially microscopic. In syphilis, a progressive fibrosis takes place, which in some cases extends through the entire myocardium. The fibroid heart is the ultimate outcome of all cases of latent syphilis. The striking feature of the fibroid area is the dilatation of preexisting capillaries or veins or a new formation of such in the fibroid area. Such areas often appear cavernous or sinusoidal. The vascular proliferation appears to be one of the distinct features of syphilitic myocarditis. In more acute cases, the myocardium is edematous, often giving a slight reaction for mucin. In a great majority of cases, the myocardium shows healed fibroid areas in association with the active infiltrations. Warthin further stated that the essential lesion is the interstitial myocarditis characterized by lymphocytes and plasma cells infiltrating along the blood vessels between the muscle fibers. The infiltrations usually are patchy or diffuse, very rarely focal or circumscribed, thus differing from those caused by an infection with *Streptococcus myocarditis*. Polymorphonuclear leukocytes are few in these infiltrations. No eosinophils are present, but mainly histogenic lymphocytes and young formative cells. Giant cells are rare. The large epithelioid fibroblast is common. The entire cardiac wall from the epicardium to the endocardium, including the papillary muscles, may be involved in the infiltrations. In the most severe cases, infiltrations are grouped around the coronary arterioles and many reach such a size as to suggest miliary gummas. A thrombosis of the thickened endocardium overlying the fibroid patches is more frequent than aneurysmal dilatation. In regard to the presence of spirochetes, he stated that in old cases with fibrosis more or less well advanced the demonstration of spirochetes becomes a task requiring patience and determination

spread often over days or weeks. Nevertheless, they are more easily found in the heart than in any other organ or tissue.

In 1925, Warthin reported 8 cases of sudden death due to exacerbation of latent syphilitic myocarditis. In this article, he spoke of active syphilitic myocarditis, of chronic syphilitic myocarditis and of fibroid myocarditis with acute exacerbation. The microscopic features of the cardiac lesions were old fibrosis (completely healed myocarditis), subacute infiltrations by lymphocytes and plasma cells, monocytes and a predominance of polymorphonuclears. Spirochetes were found, particularly in these more acute areas. All stages of syphilitic myocarditis were represented in these lesions. Miliary gummas with giant cells were occasionally found (particularly in a certain case). The coronary changes were relatively slight, except in 1 case in which the anterior left descending branch showed extensive syphilitic arteritis. No Aschoff's nodes were found. Warthin also stated that the important, if not predominant, rôle played by syphilis in the production of myocardial incompetency is not recognized by the major part of the profession, internists as well as pathologists. Old latent syphilis is one of the most important causes, if not the most important cause, leading to myocardial incompetency. The person with latent syphilis in the great majority of cases eventually dies from "cardiac failure." This may be brought about in several ways: (1) myocardial atrophy and fibrosis due to slowly progressive, mild syphilitic lesions in the myocardium (this is the most common form); (2) syphilitic disease of the coronaries with resultant infarction and fibrosis; (3) a combination of these two processes; (4) syphilis of the aortic valve (always associated with some degree of myocardial syphilis); (5) a combination of myocardial syphilis and syphilitic mesaortitis, and (6) acute exacerbations of a previously latent syphilis ("critical stage" of syphilitic infection) or an acute malignant type of cardiac syphilis. This is much less common than the chronic latent form, but its frequency remains to be determined, as it has not received pathologic recognition.

#### ANALYSIS OF THE HISTOLOGIC CRITERIA OF SYPHILITIC MYOCARDITIS

If one regards the criteria of syphilitic myocarditis critically, so as to rule out any other etiologic agent as the basis for such lesions, the following arguments present themselves.

Myocarditis is classified as parenchymatous and interstitial. Though such a subdivision is not always clearly distinguishable, it can be made in many cases. In cases of interstitial myocarditis, the inflammatory cells are found close to the branches of the coronary vessels, which always lie within the interstitial tissue. As a matter of fact, scar tissue

as the result of healed myocarditis is often confined to the perivascular spaces. It is also known that the place of predilection of Aschoff's nodules is the perivascular space of the interstitial tissue in the myocardium. A perivascular distribution of cells or of fibrous tissue in the myocardium, therefore, is not pathognomonic of syphilitic myocarditis.

It is true that the typical cells in syphilis are the lymphocyte and the plasma cell. But it is also known that in many instances of myocarditis of nonsyphilitic origin, plasma cells and lymphocytes are found. Especially the isolated form of myocarditis (Fiedler's myocarditis) shows, in addition to other cells, endothelial cells, many lymphocytes and plasma cells (Scott and Saphir). As to the finding of round cells in the myocardium, Liebmann found an accumulation of round cells in the perivascular spaces of the myocardium in two cases of pneumonia. As will be shown later, infiltrations by round cells in the myocardium are often found in cases of coronary sclerosis. The diagnosis of syphilitic myocarditis, therefore, should not be based alone on the finding of these varieties of cells.

A diffuse fibrosis of the heart is also found in other conditions. Rheumatic myocarditis results often in diffuse fibrous patches throughout the myocardium. After scarlet fever, typhoid fever, pneumonia and other nonspecific infectious diseases the myocardium may show a diffuse fibrosis. The cases reported by von Glahn and Wilshusen are excellent demonstrations of the coincidental finding of syphilitic aortitis and rheumatic myocarditis in the same case. Huebschmann described marked fibrosis of the myocardium following diphtheritic myocarditis; the fibrosis was so extensive as to be called "cardiac cirrhosis." Similar cases were reported by Schmincke. Besides, it should not be forgotten that chronic toxic processes such as chronic alcoholism, gout, lead poisoning, etc., may lead to chronic interstitial myocarditis (Karsner).

The young granulation tissue often described as occurring in syphilitic myocarditis also is not pathognomonic of syphilis. It is found in cases of coronary sclerosis, and more often in cases of subacute myocarditis occurring in the course of subacute bacterial endocarditis. But it also seems evident that young granulation tissue might easily be found in any case of myocarditis of longer duration.

Sclerosis of the coronary arteries, such as was present in some of the cases recorded in the literature, might have been the cause of some of the fibrosis ascribed to syphilitic myocarditis. Kaufmann mentioned the richness in blood vessels of the granulation tissue in coronary sclerosis. He stated that such fibrosis might be diffuse and therefore spoke of "myocarditis fibrosa disseminata." Karsner also spoke of fibrosis due to progressive arteriosclerosis. The larger branches of the coronary arteries might be practically free from changes while the smaller branches show severe lesions. As was mentioned before, steno-

sis or occlusion of the mouths of the coronary arteries might lead to fibrosis of the myocardium. Such conditions are found especially frequently in cases of syphilitic aortitis. Moenckeberg mentioned that changes in the myocardium sometimes are found in cases in which the coronary arteries offer no changes, and in which only the ascending portion of the aorta is the seat of sclerotic lesions. Moenckeberg stated that a rigidity of the ascending portion of the aorta alone might explain the insufficient blood flow through the coronary arteries and hence myocardial fibrosis. It is known, however, that in some cases a constriction of the mouths of the coronary arteries does not necessarily lead to marked myocardial changes. But there are also cases on record which show myocardial infarction referable only to constriction of the mouths of the coronaries in cases of syphilitic aortitis. Areas of myocardial infarction show, in addition to necrosis, a cellular infiltration, especially of polymorphonuclear leukocytes, as was shown recently by Wearn. Such a case will be mentioned later.

It is true that endarteritis obliterans is very characteristic of syphilis, but by no means pathognomonic. It also is observed in chronic inflammations of nonspecific origin. Besides, in the cases recorded in the literature in which the diagnosis of endarteritis was made, it does not follow from the descriptions that this was really primary endarteritis obliterans and not merely the end-stage of arteriosclerotic intimal thickening in small vessels resulting in obliteration of the lumina.

Fatty degeneration, atrophy, necrosis and edema are not characteristic of syphilis. In view of the finding of necrosis, it might be mentioned that Bracht and Waechter have shown such an occurrence in their experimental studies with intravenous injection of diplococci and streptococci. Kaufmann mentioned finding necrosis and fatty degeneration of the myocardium in cases of acute interstitial myocarditis. Edema and mucoid degeneration were described in a previous paper (Saphir and Scott) as resulting from vascular changes, and as such they are not characteristic of syphilis.

In the cases of syphilis with gummas in the myocardium, or at least accumulations of cells suggesting miliary gummas, with giant cells, the syphilitic nature of the myocarditis seems established.

Summarizing the histologic criteria of chronic syphilitic myocarditis, I must state that none of the criteria is sufficient to allow the making of such a diagnosis. I do not believe that without knowing the history of the case and without being told that the Wassermann reaction was positive, or that the patient had other signs known to be those of syphilis, the diagnosis of syphilis could have been made from the histologic lesions of the myocardium alone in any of the cases reviewed.

## SUMMARY OF EVIDENCE AGAINST THE DIAGNOSIS OF SYPHILITIC MYOCARDITIS

Hertz as early as 1873 doubted the diagnosis of syphilitic myocarditis in the case that he reported, even though an aneurysm of the aorta and so-called syphilitic pneumonia were present. Ziegler in 1887 stated that many cases reported in the literature as cases of syphilitic fibrous myocarditis are more likely cases of myocardial fibrosis on an arteriosclerotic basis. Curschmann mentioned that to the time of his report in 1893 there were no definite criteria allowing a differentiation between syphilitic proliferation of connective tissue and fibrosis from other causes. Stolper preferred to speak of fibrous myocarditis in syphilitic persons rather than of syphilitic myocarditis. Quensel remarked that fibrous myocarditis found in the heart in cases of syphilis offers nothing characteristic of syphilis either grossly or histologically. Berblinger was of the opinion that histologically one cannot differentiate between simple fibrous myocarditis and fibrous myocarditis as the result of gummatous myocarditis. In the discussion of Benda's paper it was brought out that every author had confessed that he was unable to find specific signs of syphilitic myocarditis. Bloch stated that no pathologist is able to make the diagnosis of syphilitic fibrous myocarditis in a given section. Moenckeberg and also Scott were not convinced that a fibrous syphilitic myocarditis exists. Stokes stated that postmortem studies have given to syphilis of the aorta a definiteness that unfortunately does not apply to gross syphilis of the myocardium. Cowan and Faulds recently stated that the ultimate lesions in the cardiac muscle are not syphilitic, though they have a syphilitic cause; that is, they are the result of occlusion of the mouths of the coronary arteries.

Ehrlich, Orth, Mracek, Kockel and Krehl believed that in syphilis primary vascular changes may lead to the histologic picture of fibrous myocarditis. Sears also stated that syphilis of the heart is essentially syphilis of the blood vessels. Brooks stated that in 35 of his cases of syphilis the changes of the coronary arteries were relatively greater than the general arterial changes. On the other hand, in my opinion, Brooks offered no proof that these changes were syphilitic.

Without discussing clinical reports of syphilitic myocarditis, I must mention that in many instances anatomic reports refer to such cases as if they were proved cases of syphilitic myocarditis. In the great majority the clinical diagnosis is based mainly on a positive Wassermann reaction and an apparent improvement of the patient's condition in the course of antisyphilitic treatment. It also might be mentioned in this connection that many articles labeled "cardiovascular syphilis" refer only to syphilis of the aorta and of the aortic valve and do not



mention involvement of the myocardium. But such a title is apt to create an erroneous conception in regard to the frequency of myocardial syphilis.

One also finds in the literature repeated statements based entirely on clinical evidence, indicating that a syphilitic myocardial lesion may occur without valvular mischief and sometimes without thickening of the superficial arteries. Such statements might be correct from a clinical point of view, but unfortunately are often misunderstood and taken as though proved anatomically.

Brooks maintained that one must be careful in asserting that no specific lesions are present in the myocardium, simply because neither gross nor microscopic changes characteristic of syphilis can be demonstrated by the usual technic. It must be emphasized, however, that the only possible way of recognizing syphilis in the myocardium is by a histologic examination. If such a diagnosis cannot be made by the use of the routine staining methods, one has no right to speak of syphilitic myocarditis, even though other manifestations of syphilis are present. A possible myocardial failure in such cases must be explained in some other way.

It seems unwarranted to base the histologic diagnosis of syphilitic myocarditis in part on clinical findings, as was brought out in the discussion of Maher's paper when Maher, asked if he thought that he could make a diagnosis (of syphilis of the myocardium) from his observations in the absence of a history of syphilis, answered that he did not think he could.

Christian, in discussing Lemann's and Mattes' paper, made the following statement:

I think that at the present time incorrectly we are regarding them (syphilitic changes) as extremely frequent. . . . One of the most difficult diagnoses before the Wassermann reaction, when the spirochete was not discovered, was a positive diagnosis of syphilis either with the naked eye or microscope. We ought to raise the question whether we are very much better off today after we have had the Wassermann reaction and found spirochetes in association with certain cell reactions in determining what is or what is not syphilis on the sole basis of observing this cell reaction. Though sometimes present in syphilis, it does not follow that syphilis is the sole and only cause. . . . It does not seem to me that the evidence can be accepted as satisfactory of the diagnosis of syphilis. . . . I do not believe a dead patient more frequently has syphilis than a live patient. . . . When we come to cardiac disease, as chronic myocarditis, we find the same incidence of positive Wassermann reaction as in the average for all of our patients: but we do not find spirochetes, except Dr. Warthin. Nobody else does with any degree of frequency in these chronic hearts, and in my judgment, the bulk of the cardiac cases, not associated with aortic insufficiency are not syphilis, and that makes up a very large percentage of the cardiac cases in people over 45 years of age. Nearly everybody who has a valvular lesion of the ordinary

rheumatic or bacterial type, dies before he reaches 45. The other cardiac cases develop later in life and are nearly always myocardial in character and very rarely syphilitic.

It also might be of interest to quote Thorel, who said that it must be admitted that the fibrous myocarditis in cases of syphilis offers nothing characteristic. It is impossible to prove in a given case that the fibroid myocarditis is syphilitic. The author believed that it is a matter of speculation and not of science to designate scar tissue in the heart, even though marked, as syphilitic simply because it is found in cases of syphilis.

The failure to find syphilitic lesions in the heart anatomically in cases in which they were apparently suspected led Renvers to the conclusion that syphilis of the heart may heal without leaving pathologic anatomic residua.

There also are authors who believe that the knowledge of histology and of syphilis is at present insufficient to rule out syphilis as the etiologic agent of the myocardial changes. Curschmann was mentioned before. Loomis stated that as knowledge of syphilitic manifestations becomes more perfect, syphilis will be recognized as an important etiologic factor in the production of lesions in the heart. Grassmann stated that the reason why syphilitic lesions other than gumma are found so rarely in the heart is that there is a lack of recognition of such occurrences. Reid stated that it is disappointing to note that in but 1 of 7 reports of cases in which syphilis appeared to be the cause of the changes in the heart did the pathologist definitely state that the lesions were due to syphilis; in the remainder the description is purely anatomic and contains no comment as to the etiology. Reid apparently did not realize the difficulties that confront the pathologist in regard to syphilitic myocarditis. An anatomic description of such changes without comment as to the etiology is practically all that the pathologist can offer.

There are several reports in the literature indicating that syphilitic changes in the myocardium are not encountered more frequently because sufficient studies are not made and not enough sections are taken from the heart for histologic investigations. Though such an argument does not seem fair, it is often used in the discussion of negative results. Sears remarked that syphilitic lesions in the brain, especially vascular changes, are encountered much more frequently than in other parts of the body because they happen to have been studied more carefully. Lamb stated that syphilitic changes in the heart are not found more frequently because it is unusual to take more than one or two pieces of the cardiac muscle for study. This may be true for the particular laboratory from which Lamb took his material, but such statements

are wrong as generalizations. Warthin, in discussing Clawson's paper, believed that the failure to find evidence of syphilis in the myocardium lies in the insufficient number of sections cut from the myocardium.

#### FINDINGS OF THE SPIROCHETES IN SYPHILITIC MYOCARDITIS

In practically all of his reports of cases of syphilitic myocarditis, Warthin mentioned the finding of spirochetes. Some of the photomicrographs of spirochetes in these articles present apparently typical spirochetes, while others are doubtful. Lemann and Mattes, Spalding and von Glahn, Hines, Boyd and Wilson also found spirochetes in the heart in cases of acquired syphilis. The photomicrograph of spirochetes accompanying the articles by Boyd and by Spalding and von Glahn are not convincing. Lemann and Mattes, and Wilson, do not show pictures of spirochetes in their articles. Spirochetes that are described as drawn out or as broken up or as remnants cannot be regarded as typical spirochetes. If nothing else is found but remnants of spirochetes, they should be entirely disregarded. Similarly, a description of a cycle of spirochetes such as was recently reported by Warthin, convincing though it may sound, should not, in this day, be taken as a description of spirochetes, and the diagnosis of spirochetes should not be based on such metamorphosed organisms. If the finding of such remnants is taken as a positive finding, the literature quickly will be swamped with reports of the observation of spirochetes in almost every organ in every disease. How easy it is to produce artefacts resembling spirochetes or metamorphosed spirochetes will be shown later.

It must be emphasized in this connection that few pathologists are able to find spirochetes in the myocardium in acquired syphilis, in spite of the fact that it is so easy to find them in the myocardium in congenital syphilis. The methods used in staining the spirochetes in congenital syphilis are irrelevant; spirochetes are found in organs in cases of congenital syphilis by the use of any method. According to Christian, nobody, however, finds spirochetes in the myocardium in cases of acquired syphilis as regularly as Warthin did.

Warthin not only found the spirochetes in the myocardium in syphilis but also stated that they are found more easily in the heart than in any other organ or tissue. The discrepancy between his observations and those of other investigators is striking. It probably does not depend on the number of sections examined and the diligence with which the investigators search for spirochetes, but on the purity or impurity of the stains used,<sup>1</sup> on the exact concentration of hydrogen

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1. J. Erdheim, in a personal communication, mentioned that the failure of Warthin's assistant to demonstrate spirochetes in his material in Vienna might be due to the fact that too pure a staining material was used.

ions in the solution used (as was demonstrated by Farrier and Warthin) and on the type of material and its relative age and preservation. Also should be mentioned the difficulties encountered in the differentiation in sections between *Spirochaeta pallida*, fusiform organisms, *Spirillum dentium* and, sometimes, artefacts.

It is also of interest to note that the finding of spirochetes in the heart in acquired syphilis is apparently little referred to in the German literature or in the French literature. Bloch in 1923 stated that spirochetes have never been demonstrated in the heart in acquired syphilis. Kirch stated that to 1927 a possible syphilitic origin of fibrous myocarditis in cases of syphilis had not been proved by demonstration of the spirochetes. Lenoble stated that it is possible that the spirochetes might disappear from the myocardium and therefore cannot be demonstrated. For that reason he spoke of "myocardites déshabitées."

Coombs made the following statement:

We have been told by some observers that this (a few plasma cells and lymphocytes drowned in serum which gives the tissue a dropsical appearance) is an inflammatory reaction excited by spirochetes lying latent in the cardiac wall, and as this is a point on which none but the most experienced observer can offer an opinion, I called on Professor Levaditi last autumn at the Pasteur Institute. He was kind enough to undertake the examination of portions of two hearts in which we had already found aortitis and myocardial fibrosis on a generous scale. He reports as follows: "We have examined the blocks that you sent, by the silver impregnation method, that up till now has given us the most reliable results. We have found it impossible to demonstrate spirochetes, in spite of the presence of aortitis. We shall continue to search and will report if we find anything positive." Coming from so experienced an authority, this verdict has peculiar weight; it is, at all events, clear that if spirochetes are in the myocardium, they are so few as to be very difficult to find.

#### AUTHOR'S OBSERVATIONS

This study deals with an examination of the myocardium in 130 hearts which, at autopsy, showed syphilitic aortitis and an extension of the syphilitic process to the aortic valves. The material was taken in part from the Cleveland City Hospital and in part from the Department of Pathology of Michael Reese Hospital; a few hearts were received from the Cook County Hospital. Blocks of myocardium were taken from various portions of each heart, but especially from the apical portions of the left ventricle and the adjacent septum. At least 15 blocks were taken; but in many cases as many as 60 or 70 blocks were examined. The sections were stained routinely with hematoxylin-eosin. The Levaditi and Warthin-Starry stains were applied for spirochetes. The age of the patients varied from 23 to 65 years. The age of the majority of the patients was about 40 years.

*Gross Appearance of the Myocardium.*—The myocardium in many cases showed no gross changes. In some cases small grayish dots

and streaks of fibrosis were demonstrable in the myocardium, while only a few cases revealed larger, pearly white plaques of fibrosis. One of the cases, that of a 29 year old woman who died suddenly, revealed a recent infarction of the myocardium, involving the apical portion of the left ventricle and extending into the adjacent septal portions. The endocardium in this region was covered with a recent thrombus. There was an early fibrinous pericarditis corresponding to the infarcted area. The mouth of the left coronary artery was completely occluded, and the mouth of the right coronary was markedly encroached on, but no thrombus was found in any of the coronary branches.

None of the cases showed gummas in the myocardium.

In 41 cases the mouths of the coronary arteries showed constriction. In 11 cases, the constriction was confined to the orifice of the right, and in 10 to that of the left, coronary artery. In 20 cases, the mouths of both coronary arteries were encroached on. In 4 cases there was complete obliteration of the mouth of the left coronary artery, but only in 1 case was there complete obliteration of the mouth of the right. The coronary vessels themselves showed a varying amount of arteriosclerosis—simple intimal thickening, hyalinization and calcification; but there was no gross evidence of syphilis seen throughout their course.

All of the hearts were hypertrophic. The hypertrophy is easily understood on taking into consideration that only those hearts were used that showed in addition to the aortitis, a syphilitic involvement of the aortic valve with insufficiency of this valve.

*Histologic Changes in the Myocardium.*—The cardiac muscle fibers were larger than normal. The nuclei showed square ends. The striations of some of the fibers were obscured. In some fields a moderate amount of fatty degeneration was found, while others showed some edema between the cardiac muscle fibers, but more pronounced in the perivascular spaces. Many hearts showed a varying amount of connective tissue, in part interrupting the course of the cardiac muscle fibers and in part surrounding branches of the coronary vessels. In many places, the fibrotic areas were very poor in nuclei and showed only a few spindle-shaped cells and an occasional lymphocyte. In other portions, the fibrosis was apparently younger, showing more connective tissue cells and a few blood vessels with indistinctly outlined walls. Other sections revealed typical organization tissue with many newly formed blood vessels, many lymphocytes, endothelial cells and a few polymorphonuclear leukocytes. In some of the hearts in which the mouths of both coronary arteries were found to be encroached on, a progressive fibrosis was noted, with pictures varying from that of early necrosis to that of organization tissue and that of old fibrotic plaques. In a few cases, some of the sections revealed a basic staining of the con-

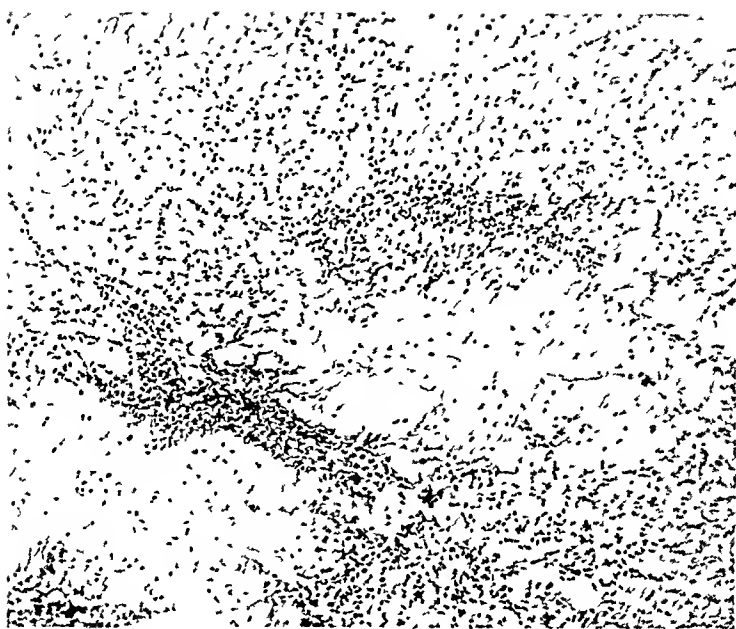


Fig. 1.—Note the polymorphonuclear leukocytic infiltration surrounding an infarcted area of the myocardium (syphilitic occlusion of the mouths of both coronary arteries). Iron-hematoxylin-eosin preparation;  $\times 80$



Fig. 2 —Polymorphonuclear leukocytic infiltration close to an early infarction of the myocardium (syphilitic occlusion of the mouths of both coronary arteries). Iron-hematoxylin-eosin preparation;  $\times 80$

nective tissue and a blue interfibrillar substance that could be interpreted as mucoid degeneration. In these portions a few stellate cells were found. Several cases showed a slight, but distinct, perivascular infiltration of lymphocytes about the branches of the coronary arteries. Typical endarteritis obliterans, however, was not observed. Sometimes a more cellular infiltration was found, pronounced mainly in the interstitial tissue. Lymphocytes, plasma cells, endothelial cells and an occasional polymorphonuclear leukocyte were present. The case described before, that of the 29 year old woman, which grossly showed evidence

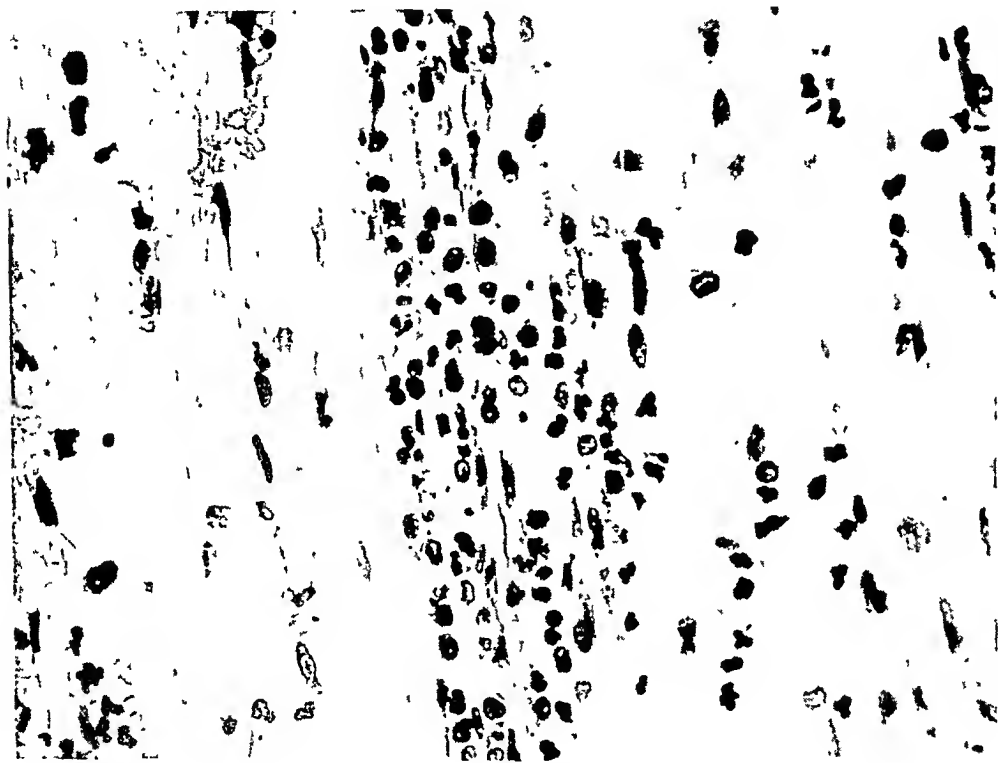


Fig 3—Polymorphonuclear leukocytic infiltration surrounding an early infarcted area of the myocardium (syphilitic occlusion of the mouths of both coronary arteries). Iron-hematoxylin-eosin preparation;  $\times 380$ .

of an infarction of the myocardium, revealed, on section, in addition to the recent necrosis of the muscle fibers, many areas of infiltration by polymorphonuclear leukocytes between which a large number of lymphocytes and endothelial cells were present (figs. 1, 2 and 3). No relationship could be demonstrated between the degree of stenosis of the mouths of the coronary arteries and the changes in the myocardium. In a few cases that showed encroachment on both mouths of the coronaries, the myocardium revealed no changes of note.

With every section of the myocardium in these cases which was stained for spirochetes, tissue from a case of congenital syphilis was

stained simultaneously as control. The control section was examined first, and only when spirochetes were found in this control tissue, were the sections of the myocardium of the cases reported here searched for spirochetes. In none of the sections of the myocardiums of the 130 hearts did the Levaditi or the Warthin-Starry stain reveal typical

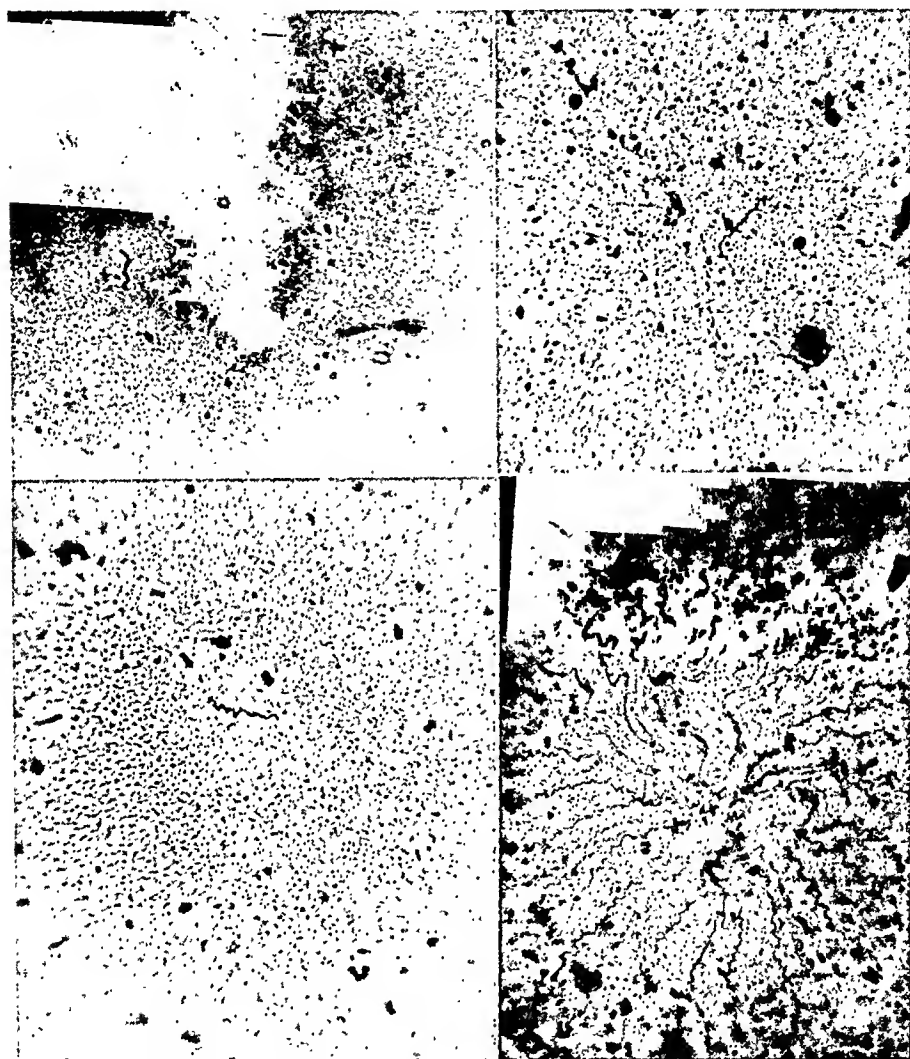


Fig. 4.—Artefacts in coverslip preparation without tissue; Warthin-Starry preparation.

spirochetes, even though in some of the cases sections from 50 different blocks were examined and spirochetes diligently sought.

After this failure to demonstrate typical spirochetes in the sections, it was thought wise to examine coverslips prepared according to the Warthin-Starry method, but without the tissue. In a number of such



slides, it was possible to demonstrate artefacts that somewhat resembled spirochetes. Figure 4 shows some of the artefacts. Similar artefacts were also produced by the use of the Levaditi stain, but much less frequently.

#### COMMENT

The morphologic changes in the cases on which this study is based were, at least, not characteristic of syphilis. All the changes could be interpreted as the result of coronary sclerosis or as following encroach-

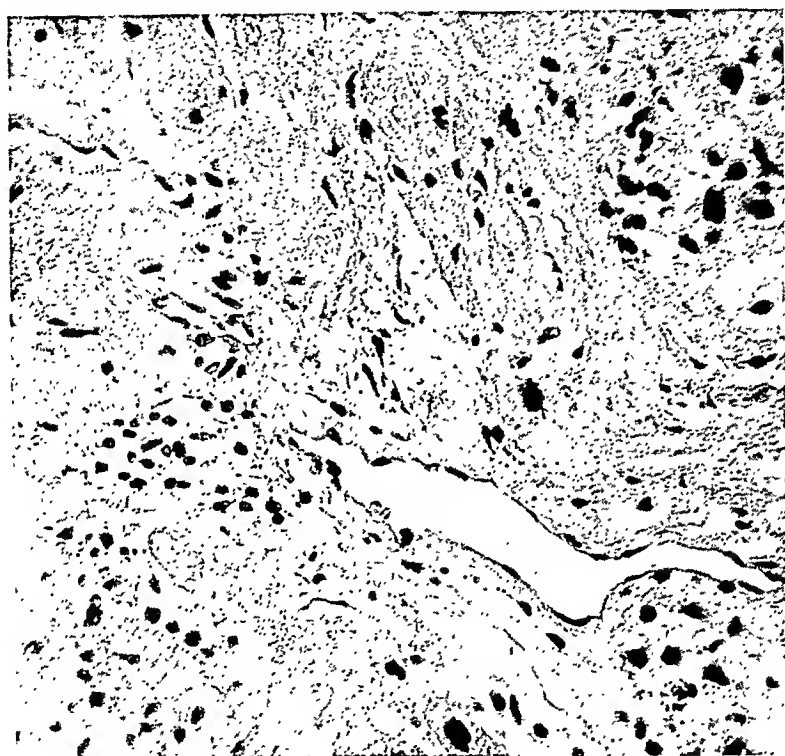


Fig. 5.—The myocardium in a case of marked coronary sclerosis. Note the perivascular infiltration of lymphocytes. Iron-hematoxylin-eosin preparation;  $\times 260$ .

ment on the mouths of the coronaries. Even though some of the cases showed a perivascular infiltration of lymphocytes in the myocardium, and even though in some of the cases lymphocytes, plasma cells and endothelial cells were found in the interstitial tissue, there were no grounds for believing that these lesions were syphilitic. Similar changes were found in the myocardium in cases of coronary sclerosis, cases which were without a history of syphilis and which at autopsy showed no evidence of syphilis. Figures 5 and 6 show myocardial areas in these control cases. It is of special interest to note that the case that showed a recent infarction of the myocardium revealed histologically

many polymorphonuclear leukocytes in addition to necrosis, lymphocytes and endothelial cells. The presence of polymorphonuclear leukocytes in the myocardium in cases of syphilis has been interpreted by some authors as significant of active syphilis. On the other hand, polymorphonuclear leukocytes were demonstrated by Wearn in infarcted areas of the heart, and are therefore as such not characteristic of syphilis.

#### SUMMARY AND CONCLUSIONS

A review of so-called chronic syphilitic myocarditis is given and the criteria of syphilitic myocarditis as recorded in the literature are

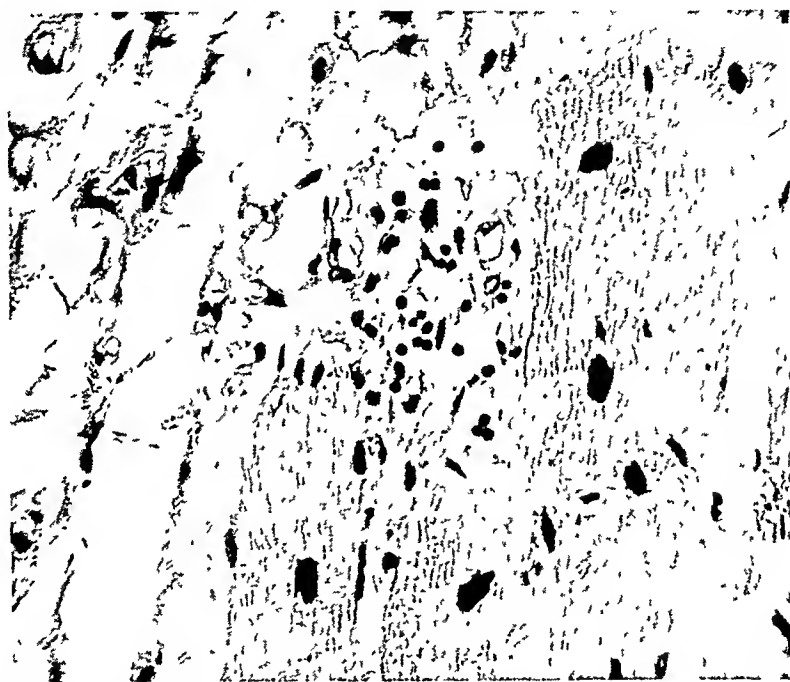


Fig. 6—Myocardium in a case of coronary sclerosis. Note the perivascular infiltration of lymphocytes. Iron-hematoxylin-eosin preparation;  $\times 380$ .

discussed. A critical consideration reveals that morphologically the diagnosis of syphilitic myocarditis cannot be made in any of the reviewed cases without the presence of gummas.

In a study of 130 cases of syphilitic aortitis with insufficiency of the aortic valve, the myocardium showed no changes that could be interpreted as syphilitic myocarditis. All the changes found in these hearts might be encountered in other conditions. Spirochetes could not be demonstrated in any of the 130 hearts. Coverslips without tissue, prepared according to the Warthin-Starry method, revealed artefacts that resembled spirochetes.

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# THE ETIOLOGY OF CANCER

## IV. CANCER METABOLISM \*

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### OXIDATION

That cancer might be the result of impairment of oxidative metabolism was suggested by van den Corput in 1883. The idea was elaborated in some detail by Wakefield in 1902; according to his view, the normally acid products of cellular oxidation require a certain degree of alkalinity for their neutralization, and in the absence of this, catabolic changes are retarded. Cancer, he believed, occurred in circumstances in which there was this retardation of catabolism either by local acidosis or by other causes of reduced oxidation, while conditions were still suited to maintain anabolic activity. In a study of the metabolism of cancer published in 1914, Braunstein was unable to find any constant change of oxidative activity in malignant tissues, and Burrows in 1917 found that availability of oxygen had little influence on growing tissues, as chick embryonic tissues were capable of growth in atmospheres ranging from pure oxygen to partial pressure of that gas as low as 45.6 mm. of mercury. With pure oxygen, however, there was some acceleration of the rate of growth—a finding that was confirmed in 1920 by Russell and Gye. More definite indications of an association between oxidative metabolism and cancerous growth were reported in that year by Russell and Woglom, who found that in mouse tissues there was in general a rise of respiratory quotient with increasing rate of growth. In the case of slowly growing tumors Russell later found that there was a low respiratory quotient, which, however, in the presence of a direct supply of dextrose became greater than unity.

Studies of the oxidative ferments of cancerous tissues have given somewhat variant results. Hugoncq and Paviot found in the rapidly growing portion of malignant tumors an increased content of these ferments; Harden and MacFayden were able to find in the juices expressed from human cancers, catalases and oxidases; Buxton found peroxidase present as in normal tissues, but could not definitely establish the presence of oxidase. As concerns catalase more specifically, Jolles

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and Oppenheim found that in the blood of cancerous subjects this might at times be normal, at times greatly reduced in amount; Blumenthal and Brahn, that there was less of this ferment in cancerous than in normal livers, particularly as regards actually cancerous nodules, but with associated diminution in the uninvolved portions of these livers as well. Rosenthal, in mice with subcutaneous tumor implants, could not find any abnormality in hepatic catalase, but in animals in which implantation had been made intraperitoneally, it was markedly reduced both in the blood and in the liver. Rhodenburg could find no constant variations in the blood catalase content in cancerous patients—a finding which has been confirmed recently by Olchowskaja and Bestschinskaja. Lewis and Corsman found in the Rous sarcoma very little catalase, but more in rat tumors; a result which they believed might have been due to associated infection. Gröbly would attach much importance to the oxidative ferments of purine base metabolism, in part because of the nucleoprotein metabolism concerned with cellular growth, in part because of their abundance in certain internal secretory organs, and he regarded a pathologic increase in nucleoprotein metabolism as a constitutional anomaly predisposing to cancer. Investigation of these particular ferments has shown no very striking results. Saiki found in cancers both guanase and xanthin-oxidase, with an absence of adenase, and Wells and Long found that in purine base content and in that of purine enzymes cancerous tissues differed little from normal ones. That, however, there may be abnormal fermentative activity in cancer with respect to oxidative activity was shown by Alsberg, who found in a metastatic melanosarcoma a ferment capable of oxidizing pyrocatechin into a melanin-like substance. Less specific investigation of the oxidative ferments of malignant tissues, as made by Drew with reference to the reduction of methylene blue, showed a lessened rate of this reduction, indicating a low avidity of tumor tissues for oxygen, a finding which has been confirmed by Heinlein and by Menten, and which is in direct contrast to the results of Shearer's study of the fertilized sea-urchin's egg, in which he found that immediately after fertilization there was a greatly increased rate of oxygen consumption. Using microchemical methods, Roskin found in tumor cells three different relations to oxidation—that of cells showing little variation from normal, in which the nucleus is the site of oxidative, the cytoplasm of reducing, activity; that of other cells with this relationship reversed, and that of a third type of cells in which oxidation occurs throughout both nucleus and cytoplasm.

Neuschloss found that the serum of cancerous individuals greatly increases the respiratory activity of normal cells; as concerns their tissues, he found that there is a general diminution of reducing power,

to which rule the spleen is an exception; but the reducing power of malignant tissues was not affected, as was that of normal tissues, by the addition of hydrocyanic acid, and it was not increased to the same degree as with normal tissues by the addition of iron salts. Händel and Tadenuma also found marked impairment of cellular oxidation in rats with large malignant tumors. Remond, Sendrail and LaSalle found that the basal metabolism of tarred rabbits increases during the pre-cancerous stages, but diminishes with the onset of actual cancer. While they would associate these changes with hypothyroidism from intoxication, they could even more probably result from general alteration of cellular oxidative activity. Sehrt observed that differences in the oxygen-combining power of blood, as detected by the addition of *alpha-naphthol* and *dimethylparaphenylendiamine* bases with and without the addition of potassium ferricyanide, are considerably less in cancerous blood than in normal. That the relations of tumor growth to oxidative metabolism would appear to be of secondary importance is indicated by the work of Wind, who found that Rous tumor cells would grow freely for as long as 48 hours in almost strictly anaerobic conditions if dextrose was present, whereas no growth took place even in aerobic conditions if it was lacking. Fischer and Buch Anderson found that cultured tumor cells could be killed by excessive oxygen tensions, malignant (sarcoma) cells in mixed cultures succumbing more rapidly than the normal connective tissue elements. With the reversed relation of reduced oxygen tension, Mottram found that sarcoma cells were capable of activity at much lower oxygen concentrations than normal cells, short of actually complete anaerobiosis. These results were confirmed by Wright, who found that while chicken heart myoblasts required a minimum oxygen tension of 12 mm. of mercury to permit cell division, the Jensen rat sarcoma could proliferate at 6 mm., and a mouse carcinoma at 3 mm. of mercury pressure.

Barry, Bunbury and Kennaway investigated the possibility of accounting for the cancerogenic power of arsenic on the basis of interference with the process of cellular oxidation; while the salts of arsenious acid were found to interfere with a number of reduction systems, this was not true of arsenates, which have been shown by experiment to be equally effective in inducing malignant growth, and they were forced to conclude that the effects of arsenic compounds in this direction were indirect, through the accumulation of organic compounds absent in the normal cell.

Among the more detailed theories that would attempt to explain the phenomena of cancer by abnormalities of intracellular oxidation is that of Bristol, who believed that the essential change is one of ferment excess, especially excess of oxidases, possibly brought to the site

of the new growth by migrant leukocytes, with resultant speeding up of biochemical reactions; the formation of lactic acid in malignant tumors would be explained by local oxygen starvation caused by the increased demand of the neoplastic cells for oxygen. Another of the theories of this character is that of Stoltzenberg and Stoltzenberg-Bergius based in part on the work of Saxl, who had found that in advanced cases of cancer, at least, there was an intermediate disturbance of protein metabolism, with excessive formation and elimination of incompletely oxidized protein waste products. In these circumstances, according to Stoltzenberg, the weakening of oxidative ferment action would be most manifest at the expense of the aromatic amino-acid compounds, and there would be an accumulation of these, which in general are cancerogenic, as is the case with indol and skatol, and less certainly with quinone. Burrows regarded his theoretical growth-stimulating substance, archusia, as an oxidation product, which in circumstances of stagnated circulation is accumulated in tissues destined to become neoplastic. Handley would see in diminished oxygen supply one of the factors that induce cancer, along with hypernutrition and reduction of hormones, all results of lymphatic stasis. Perhaps the most carefully elaborated of these theories is that of Brach, who differentiates between active and inactive oxygen, the former of which alone is capable of supplying cellular necessities. Activation is accomplished by means of hormones and vitamins, and probably also by certain mineral substances of vegetable origin. Insufficiency of these, or diminution of "activated" oxygen by the accumulation within cells of abnormal reducing substances, forces the cell to rely principally on the aerobic cleavage of dextrose for its principal source of energy. The lactic acid formed by this may lead to hydrolysis of proteins, or possibly to lowering of surface tension, with increase of cellular nutrition and further suppression of oxidation; in these circumstances, in the presence of an added element of irritation, there is an absence of normal inflammatory reaction and the products of cellular disintegration contribute to the material at hand for the growth of the cancer cells.

#### REACTION

Among the earlier works that furnished the suggestion that the phenomena of malignant growth might rest in part at least on a basis of altered reaction was that of Jacques Loeb, who found that the immersion of the larvae of *Arbacia* in weakly alkaline solutions accelerated their development and growth, while immersion in weak acids had the reverse effect—results which he explained on the grounds of acceleration of oxidation in the one case, and retardation in the other. In 1905 Moore, Alexander, Kelly and Roaf reported that in cases of malignant disease, regardless of the location of the lesion, there was a reduction of total

gastric acidity, which they interpreted as being the result of diminished hydrogen ion concentration in the blood, with relative increase of hydroxyl ions, giving a condition of relative alkalinity similar to that in which Loeb had observed acceleration of growth. The same workers reported effects similar to those previously observed by Loeb, in studies made on the fertilized eggs of the sea-urchin, and found that with the addition of small amounts of alkali to the medium in which the eggs were immersed, there followed not only an increased rate of growth, but also the appearance of irregularities in the size and shape of the cells, and at times atypical mitotic figures. Their findings in regard to gastric secretion were not confirmed by Copeman and Hake, who found in the stomachs of cancerous mice an increased acidity. The theory of alkalinity as a factor in the induction of cancer was again advanced by Hertzler, who regarded the disease as originating at sites where there was a combination of irritation and exposure to alkaline secretion. McClendon and Mitchell, reasoning again from the changes induced in the developing sea-urchin's egg by alkalization, regarded increased cellular permeability as an important adjunct to the process, as with such a change incident to cellular fertilization or parthenogenetic development, the increased production of carboxyl radicals and their free escape from the cells would lead to their substitution by hydroxyl, with increased oxidative metabolism as the result of the latter change. Menten, investigating the matter of possible alkalinity in cancer as this might be reflected in the blood, confirmed Moore and his co-workers in reporting that in cancer there was a relative alkalosis, though this was not specific to the disease.

The observation of Benedict that between 75 and 80 per cent of gastro-intestinal cancers occurred at sites of the elimination of acid could be interpreted as in accordance with these views, although it was to the acid character of the surface reaction that his attention was directed. Ahlgren, in a study of tumor metabolism published in 1923, again emphasized the importance of reaction as a determining element in respiration, but hesitated to draw any conclusions, the more particularly since he found some evidence that suggested that actually there was a lesser intensity of respiratory activity in tumor tissues than in general in normal tissues, his basis for comparison being muscle.

Theoretical considerations of the importance of alkalosis in the development of cancer have in more recent years been advanced by Rohdenburg, who would postulate the process as one of repeated and successive cellular destruction, with alkalization and lysis of the dead cells; from the accumulation of mineral constituents there results a condition of local hypertonicity, and from this in turn there is an increased influx of body fluids and local overnutrition, with increased and eventu-

ally unrestrained hyperplasia. Laville would ascribe cancer to increased oxidation in association with alkalosis, along with an upsetting of conditions of electrical equilibrium; Willy Meyer postulated alkalosis as one of the elements in his series of precancerous changes. Butts regarded the stimulation to malignant growth as essentially the development of a preponderance of positive ions within the cell—fundamentally a condition of intracellular acidosis. This theory he based on the experiment of connecting the healthy portion of an implanted Flexner-Jobling carcinoma in a rat by wire with a tumor-free litter mate, in which circumstances he observed a flow of current away from the tumor.

In a study of changes in reaction incident to the onset of cancer, Remond, Sendrail and LaSalle found that in tarred rabbits during the precancerous stages there was an actual lowering of the  $p_H$  value of the blood plasma, which approached the point of acidosis with the development of actively malignant growth, after a temporary increase of the alkaline reserve coincident with the appearance of cancerous change. Woglom, in a direct study of the reaction of tumor tissues, found that while these were more alkaline in reaction than normal muscle tissue, there was little difference in this respect between them and other normal tissues as they occur in the rat. Harde and Danysz-Michel, and Henri, have found that in transplantable tumors of rats and mice, as well as in growing embryonic tissues, there is an increased acidity, with the hydrogen ion concentration ranging from  $p_H$  5.8 to  $p_H$  6.2. Millet noted a less pronounced acidification, with an average hydrogen ion concentration of  $p_H$  6.78, as compared with a normal of  $p_H$  6.92, and Milone found in spindle cell sarcoma of the rat transplanted into the eyeball a hydrogen ion concentration of less than  $p_H$  6 within 4 or 5 days after the implantation. The reverse finding of increased alkalinity in animal tumors was reported by Goldfeder, who found in a number of neoplasms—Rous sarcoma and mouse carcinoma and sarcoma, hydrogen ion concentrations ranging from  $p_H$  7.5 to  $p_H$  7.7. However, the alkalinity was greatest in necrotic portions of the tumors; as might be expected, it was decreased by abundant supplies of dextrose. Reding, and Bischoff, Long and Hill, and Chambers and Kleinschmidt, found a relative alkalosis of the plasma in most cases of cancer—a condition that, according to the first-named, precedes the onset of the disease and is present to some degree in relatives of cancerous subjects, from which he deduces its importance not only in cancerous predisposition, but in the hereditary character of this as well. Chambers and Kleinschmidt, on the other hand, could find no evidence that the change bore a primary relationship to cancer, and Reding's conclusion was disputed by Sannie, who considered that the methods of Reding and his



co-workers were not sufficiently accurate to warrant conclusions so absolute; his interpretation of the alkalosis of cancer is that it is a compensatory mechanism.

As concerns conditions favoring growth of cancer cells *in vitro*, Fischer found that the cells are more sensitive to acidification than are normal fibroblasts, and that their growth ceases at  $p_H$  5.9; on the other hand, they resisted alkalization better than did normal cells. In connection with Gye's work on the filtrate of Rous sarcoma, Harde suggested that the former's results might be due in part to acidification of the filtrate during the course of the experiments, as Harde had found that acidification up to a certain point actually enhanced the virulence of the Rous filtrate. This finding could be confirmed only in part by Baker and McIntosh, who found that the effect of acidification was a complicated one, dependent in part on the presence of proteolytic ferments; in ordinary circumstances they observed an effect opposite to that of Harde.

#### INORGANIC CONSTITUENTS

In 1903 Braithwaite advanced the theory that an excessive intake of common salt was the cause of cancer, as in England, at least, the disease was relatively rare among the Jews, who notoriously do not consume salted pork products, and as, in his opinion, the decreased elimination of hydrochloric acid and the alleged increase of blood chlorides pointed, however obscurely, to such a relationship. Sykes promptly called attention to the fact that in normal persons excessive intake of salt is followed by its prompt elimination, and noted that in sailors of older days, forced to live in large part on salted meats, there was no noticeably greater incidence of cancer than among their shore-faring fellows; Urquhart cited several reasons why the theory should not receive any great consideration—the fact that even when salt meats played a larger part in daily diet than they do now, cancer was no more frequent, and that during a period of greatly reduced consumption of salt in Scotland, occasioned by excessive taxation, there was no reduction in the incidence of cancer. The theory was again revived by Robinson in 1917, on the basis of experiments by Carrel, which showed that tissue growth *in vitro* was accelerated by increased concentration of salt in the medium. Robinson's detailed theory was far from clear; it embraced the idea of a substitution of potassium by sodium salts, the liberation of sodium by the formation of hydrochloric acid, and the oxidation of the metallic element—a conception which it is hard to reconcile with the facts of everyday chemistry. The directly reversed view was embraced in the theory of Packard, that cancer is due to the artificial demineralization of present day food, an idea based on the alleged rarity of cancer in primitive peoples.

The idea advocated by Scherk in 1904, that cancer may result from imbalance of the inorganic elements of the body, has deservedly received more careful consideration. Bristol in 1913 suggested that the development of cancer occurred in a series of stages, the first of which was one of cellular degeneration from local causes, the second one of increased affinity of the tissues for certain inorganic salts, and the third a resultant disturbance of chemical equilibrium, which affected adjacent cells, possibly by increasing their permeability, and stimulated them to excessive and even unrestrained growth. Among the later advocates of the importance of salt imbalance as a cause of cancer, different views have been taken of its character. Dubard regarded it as one of substitution of calcium salts for those of magnesium, with a possible indirect action through the nervous system. Willy Meyer very recently regarded the imbalance as essentially one of replacement of calcium by potassium salts, brought about by a variety of mechanisms—hormonal disturbances, aberrations of the normal relations between sympathetic and parasympathetic nervous systems, etc. In these circumstances there are an alteration of cellular permeability and an alkalosis that permits cells stimulated by locally formed necrohormones to assume the property of unrestricted growth. Much the same view was taken by McDonald, who regarded the change in salts as one of substitution of the alkaline metals for the alkaline earths, with ensuing greatly increased cellular permeability and an alkalosis that in unicellular organisms is followed by increased division. Letulle and Vinay suggested that the greater death rate from cancer in northern France is due to the high content in the dietary of the inhabitants of that region of potassium salts—a result of the intensive use there of potassium fertilizers. De Raadt has rather recently reported the alleged production of cancer in four of ten mice by a diet rich in potassium salts, which in addition was of highly basic character.

Beebe in 1904 and 1905 found that in the degenerated portions of tumors there is about ten times the content of calcium salts as in the rapidly growing portions—a relationship that was reversed in progressing tumors; this finding was confirmed by Clowes and Frisbie, who found that the most rapidly growing and successfully transferable tumors were those with a ratio of potassium to calcium of from 2:1 to 3:2. Cattley in 1907 investigated the occurrence of potassium salts in rapidly growing tumors by microchemical staining reactions, and found that while in malignant tumors the intracellular distribution of these salts did not differ appreciably from that of normal cells, there was a superabundance of them in the more actively growing portions. Wolff, and Rohdenburg and Krehbiel, in 1923 and 1924, respectively, reported likewise an excess of potassium salts in actively progressing transplantable tumors, in the latter case with variations within the

scope of a single such tumor. Goldzieher and Rosenthal in a study of the effects of salts on tumor growth in 1913 reported that on the treatment of animals in which tumors had been implanted with potassium salts there was an increased rate of growth of the tumors, and restraint of this after the administration of calcium salts—a result which was confirmed by *in vitro* experiments of Cramer in 1918, who found that while the immersion of cells of transplantable mouse tumor in isotonic solutions of sodium salts was without appreciable effect, their immersion in calcium salts was followed by marked diminution of growth; the effect was transitory and was accompanied by loss of water by the cells and an appearance of increased density. Lazarus-Barlow in 1922 attempted to increase the susceptibility of rats to spontaneous development of tumors by feeding them with potassium phosphate, as well as by exposure to the x-rays, after which he attempted to induce tumors by the insertion of radium, with at most inconclusive results. Händel in 1923 again confirmed the results that had been previously reported concerning the effects of the administration of potassium and calcium salts, in that he found that animals fed with the former were not only more susceptible to implantation of tumors, but that in them the tumors progressed more rapidly, while in those fed with calcium salts the reverse was true, though only to a limited degree. He found that restriction of sodium chloride in the diet had no apparent effect, nor was there any after the feeding of phosphates. In contrast to these findings, Herly was unable to observe any differences in the behavior of malignant tissues after immersing them in physiologic solution of sodium chloride, Ringer's solution and Locke's solution. Packard found some evidence that the effects of potassium and calcium salts may be produced in part by other members of their respective chemical groups, in that with *Paramecium* the rate of division may be regulated, at least in part, by the relative proportions of sodium salts to those of calcium, although with too great disproportions there was retardation of division.

A suggestion of the mode of action of salts in influencing the rate of growth of tissues is furnished by the work of Magath, who found that imbibition by cancer cells after immersion in weak acids is greatly reduced after their preliminary treatment with calcium chloride solution—a phenomenon which is also shown by embryonic tissues, and which would appear to indicate the action as being an effect of alteration of cellular permeability. Lasnitzki would trace the inception of malignant growth to increased growth function caused by colloidal swelling produced by increased intracellular content of potassium salts, which he regards as having the added effect of favoring the hydrolytic cleavage of dextrose to lactic acid.

Delbet would see a relative deficiency of magnesium salts as an important factor in the genesis of cancer. In support of this view he adduced clinical evidence of the subsidence of precancerous lesions after the administration of magnesium salts, and, in guinea-pigs, the failure to develop of cancer-like lesions of the gallbladder after the insertion there of solid bodies, as in Kazama's experiments. Also, in a limited number of rabbits, he observed lack of development of hypertrophic lesions or of cancer after the application of coal tar. With implanted tumors in mice, he reported considerably diminished progress of these tumors under the administration of magnesium salts. In addition, he alleged that the geographic distribution of cancerous and noncancerous areas is associated with the relative absence or abundance of magnesium in the terrain, and stated that in brewery employes, cancer is exceedingly rare—a fact that he would explain by the high magnesium content of most beers. Just why cancer should be not particularly infrequent in countries with high beer consumption is not explained. Finally, he suggested that the alleged increase in the mortality from cancer is to be associated with the elimination of magnesium salts in the process of food refinement. Whatever may be thought of Delbet's views, they receive a possible theoretical support, in view of the importance of carbohydrate metabolism in malignant disease, in Canals' finding that with at least certain carbohydrate-splitting ferments—sucrase and invertase—magnesium takes the same relative importance as does manganese in the oxidases.

Studies of the systemic changes in salt content of the body in association with cancer have furnished results that on the whole are of little significance. Rohdenburg and Krehbiel considered that after the parenteral introduction of living cells, regardless of their type, there was a demineralization of the blood and general tissue cells, with concentration of these mineral elements in the actively growing cells. With the death of the cells, the salts were again returned to the general circulation. These changes, as well as those of altered ratios between various salts, they considered as purely secondary. Theis found that in cancerous serum there was a lowering of the calcium content, while that of sodium was normal, and that of potassium was somewhat lowered as a rule; Paolucci reported the occurrence of a slight rise of blood calcium early in cancer, with a subsequent decrease; Krehbiel found that in human cases blood calcium remains within normal limits. As to potassium, Ross found a deficiency of this in cancerous patients, but Ottonello reported a usual, but not constant, increase of serum potassium, although he did not find this change specific for cancer; Robin found in advanced cases of cancer a high ratio of potassium to sodium, with a reduction of calcium salts—changes which he regarded as secondary to the nutritive changes associated with cancerous lesions of bone or liver.

That the changes in salt and water content are not so much peculiarities of tumors as of growing tissues in general has very recently been indicated by Bricker and Lazaris, who found in regenerating tissues the same increased water content and excess of potassium over calcium.

There is a limited number of studies, likewise of no particular significance, in relation to other inorganic constituents of cancer cells. Tracy in 1905 could find no differences between tumor and normal cells as regards iron content; Medigrecanu, studying the presence of manganese salts in tumors because of the importance of these in relation to oxidase activity in vegetable tissues, could find no increase of these. Taverne, in a case of human cancer, found the content of copper salts reduced below that of normal tissues, and no particular differences in zinc salt content. Robin found that cancerous tissues have the power to fix to a slight degree silica, chalk and magnesia given in the food.

#### NITROGEN METABOLISM

Although much work has been devoted to the study of the nitrogen metabolism of malignant tumors, this has failed to reveal any characteristics in regard to which they are peculiar. Unquestionably many of them show increased disintegration of nitrogen compounds, as was pointed out by Müller in 1889; while in 1895 Nepveu reported finding in such tumors indol and indican, as further evidence of drastic protein cleavage. Petry in 1899 found that in cancers there was a relatively low proportion of albuminous nitrogen, although there was no constancy in the proportions of albumins to globulins; nucleoproteins, he found, were increased, and a general increase of noncoagulable nitrogen he ascribed to an increase of proteolysis beyond what occurs in normal tissues. Further support for the conception of increased autolytic activity in cancerous growth was afforded by the work of Emerson, who in 1902 reported the presence, in gastric cancers, of ferments that carried protein digestion beyond the albumose stage. Glaessner in 1903 reported the regular finding of tryptophan in cancerous stomachs, and its formation if cancerous tissue was added to normal gastric juice. However, he found that it might occur occasionally in other circumstances, as in the hyperacidity of gastric ulcer, and Erdmann in the same year found that while tryptophan was present with some regularity in cases of pyloric cancer, it was occasionally absent in that condition, and was likely to occur in almost any condition of gastric stagnation. Later findings of abnormal amino-acids in cancerous stomachs are those of Fischer in 1908, who identified tyrosine, leucine, arginine and lysine there. In 1909 Neubauer and Fischer announced finding in such cases a peptone-splitting ferment to the presence of which they attached diagnostic import, a conclusion that was disputed by Kutt-

ner and Pulvermacher, who found that such ferments were not constantly present in cancer and were occasionally present in normal stomachs. An increased content of amino-acids in cases of gastric cancer has also been reported by Barlocco. As concerns abnormal ferment activity in cancers other than those of the stomach, Blumenthal in 1905, after having previously reported his inability to find anything specific in the metabolism of cancer, stated that he had found in malignant tumors abnormal heterolytic ferments, and Neuberg in the following year announced similar results. In 1907 von Leyden and Bergell, on the basis of altered reactions of tumor tissues to various proteolytic ferments, reached the conclusion that the unrestricted growth of cancer was based on the lack in the host of certain hydrolytic ferments—that it was due fundamentally to a local aberration of protein synthesis, without however implying the necessity of the formation of any specific albuminous substances. Bergell and Dörpinghaus found, in an attempt to determine the amino-acids of cancer tissues, a high content of both mono-amino and di-amino acids, with a strikingly low content of leucine, and Bergell reported that cancerous tissues showed an unusually high content of di-amino acids, which he found constituted 40 per cent of the total nitrogen of both human and mouse tumors, as compared with a normal proportion of about 30 per cent. In sarcomas, however, he found that they amounted to only about 24 per cent. These findings of a disproportion of amino-acids Beebe could not confirm.

Hofbauer advanced a theory of cancerous growth on the basis of abnormal protein cleavage, with the view that infiltrative growth could be explained by the inability of delimiting connective tissue to withstand attack by abnormal ferments, possibly because of a deficiency on its part of antiferments. Westenhoeffer considered the cancer cell as one that had undergone such extreme reversion and modification of ferment activity as to constitute a new, essentially foreign cell. In freshly excised and nonulcerated cancers of the breast he found a content of amino-acids and albumoses absent in normal tissues, and with this evidence of increased proteolytic power on the part of cancer, he based his surmise as to the inability of the stroma to resist these ferments. Rulf in 1909 also believed that explanation of the aberrations of cancerous behavior was to be sought in differences already demonstrated in the albuminous metabolism of cancer cells.

In contrast to those findings, which indicate definite, and possibly specific, variations of protein metabolism in cancer, there is a large amount of work that indicates that the changes found are inconstant and of no general significance. Petry in 1902 found that the products of autolysis of cancer were the same as with normal tissues. Braunsstein in 1904 was unable to find any constant peculiarities in the meta-

bolic activities of cancerous tissues; no demonstrable differences were apparent in chlorine metabolism, while with 3 of the cancers studied by him there was an increase of nitrogen output. Beebe found evidence that the abnormal proteolysis frequently shown in tumors was due to circulatory impairment rather than to any fundamental variation in metabolic activity, and Buxton and Shaffer found that the intracellular ferments of tumors were closely similar to those of normal tissues, although at times there might be considerable quantitative differences. Wolff, in an attempt to determine the extent of proteolysis in the living cancerous individual, investigated the appearance of cytolytic products in the urine, with completely negative results. Labbe and Mouzaffer in a similar study observed urinary output of amino-acids regularly only in circumstances of involvement of the liver. Wolff also reported finding the proportional distribution of protein compounds in cancer directly the reverse of that reported earlier by Petry, in that his determinations showed a relative increase of the albumin fraction over the globulins. Such differences, however, as he was able to establish were at most quantitative and inconstant. The report by Brieger and Trebing, that there is an increase of antitryptic power in the serum in cases of cancer, might have lent support to the explanations of cancer on the basis of abnormal protein metabolism, were it not that they found this change a phenomena of cachexia rather than of cancer itself. Further evidence that the proteolytic abnormalities found with cancer were not specific was afforded by the work of Hess and Saxl, who could find no evidence of increased autolysis or heterolysis in malignant tumors, and that of Abderhalden, Koelker, Medigreanu and Rona, who in detailed studies of the proteolytic ferments of tumors found quantitative, but not qualitative, differences from those of normal tissues. The finding by Yoshimoto, later confirmed by Robin, that there were increased powers of autolysis both in cancerous metastases in the liver and in uninvolved portions of that organ, indicated in all probability a quantitative difference of this sort. Kepinow, too, found that mixtures of various organs and cancer tissues failed to show any appreciable alteration of proteolysis. In a later work Abderhalden and Pincussohn found differences in the proteolytic action of malignant tissues, but these were inconstant. Hamburger was unable to distinguish any difference in ereptase activity between cancer and normal tissues, and Weil, in a study of the behavior of cancerous ascitic fluids, could find no abnormalities of ferment action. Lieblein could find no evidence of constant alteration as regards autolysis and heterolysis by cancer tissues. Falk, Noyes and Sugiura, studying the ferment activities of tumor tissues, found for proteases that while there was some heightening of the activity of these, the ferments themselves were without significant differences from those

of normal tissues; nor were they successful in finding in tumor tissues substances that modified ferment activity. Robin again, in 1926, reported the discovery of highly proteolytic ferments in cancer, and Edlbacher and Merz in 1927 announced that in all the tumors studied by them there was a greatly increased capacity for arginine cleavage, but this increased arginase content they found was a feature of growing mammalian tissues in general, as it was present also in granulations, polypoid growths and embryonic tissues; with fowl tissues the findings were either less decided or negative.

Efforts at tracing abnormalities of nitrogen metabolism in cancer through changes in the circulating blood have shown that here, too, changes are inconstant, and so of little significance, quite aside from the probability of their being secondary. Theis and Stone in 1919 found that in general there was a low serum content of nonprotein and urea nitrogen, with uric acid in normal amounts. Robin in 1920 reported that there was an increase of total nitrogen, and Loeper, Thinj and Tonnet that the blood changes indicated a pronounced disturbance of nitrogen metabolism with increase of residual nitrogen and relative decrease of the urea nitrogen of the blood—a change which they ascribed to the ereptic activity of the tumor, with an added effect through involvement of the liver. Killian and Kast reported in 1921 that in 80 per cent of over 100 cases of cancer they found an increase of blood uric acid, and in 60 per cent an increase of urea nitrogen—changes that were almost certainly secondary, as they were constant in general abdominal carcinomatosis, present in 90 per cent of cancers of the lower part of the abdomen and in 50 per cent of cases of gastric cancer, and rarely present with external cancers. Theis in 1921 was unable to find any changes in the quantity of proteins in the blood plasma, while Loeper, Forestier and Tonnet reported an increase of these, and particularly of the globulins. Bucher in 1922 found by physical methods a relative increase of serum globulin—an observation which was confirmed by Kennaway in 1924, who, however, did not find this change constantly; by Galehr in 1925, and by Lewin in 1927. Both Galehr and Lewin ascribed the change to increased cellular destruction, and as further evidence to this effect Kotzareff and Weyl reported in 1923 that in cancerous rabbits there was an increased blood content of amino-acids and amines, to which they assigned a similar origin.

Some allegedly successful efforts have been made to identify specific albuminous constituents of tumors by means of immunization procedures. Loeper, Forestier and Tonnet believed that they could sensitize guinea-pigs to cancer albumins by the injection of the serum of cancerous patients, and Seyderhelm and Lampe reported that the alcoholic extraction of human cancerous material yielded a substance extremely toxic to mice, provided the extraction was conducted at



extremely low temperatures; Mertens likewise was able to secure similar toxic material from tumors; but such findings are of little exact significance in view of the known toxicity of partial protein cleavage products. Very recently Auler, Schlottmann, Rubenow, Meyer and Wolff reported that by means of a carefully prepared antigen they could cause the development of antibodies specific to tumor cells. Still more recently, Ottensooser has reported some not particularly convincing evidence of passive immunization to transplantable mouse carcinoma.

A number of abnormal nitrogenous substances have been reported as present in the urine of cancerous patients, usually with the implication that they are specific for this disease. Obviously if this were true, it would indicate definite alterations of protein metabolism; but they would appear to be products of the destruction of tissue in general rather than of an origin connected with peculiarities of the metabolism of tumor cells. Salkowski in 1905 and subsequent years reported that in such urines he was able to find large amounts of colloidal nitrogen-containing material, precipitable by alcohol or by salts of heavy metals. The substance has been variously identified—as chondroitin-sulphuric acid and compounds of nucleic acid by Sasaki and as a glycopeptide by Ebbeke. The specificity of this urinary abnormality was disproved by Ishioka, who found that material of this character might be present in small amounts only in cancerous persons and at times in large quantities in noncancerous persons; Carforio also found that the change was not specific for cancer; Mancini associated it with increased colloid content of the lymph and blood stream, and Meidner found that it was a manifestation of wasting diseases in general. Another abnormal urinary product was associated with cancer by Salomon and Saxl—a high content of oxyproteinic acids, which showed a wide variation in nitrogen content and a rather definite content of partially oxidized sulphur compounds, although in later publications they stated that these sulphur compounds appeared to have no definite relation to the oxyproteinic acid bodies. The nonspecificity of these changes was pointed out by Pribram, Kaldeck, Kahn and Murachi.

One rather constant abnormality of cancerous tissues in their relation to nitrogen metabolism is the fact that protein substances constitute in them a considerably less proportion of the total weight of the fresh tissue than is true of corresponding normal organs. Cramer and Pringle found that in malignant tumors there is a protein content only about three-fourths that of normal tissues, and that in the growth of tumors there is relatively less nitrogen retention than is necessary for somatic growth. Robin announced similar findings, which he explained in part by the assumption of especially intense proteolytic activity about, but not in, the cancerous tissues, and a resynthesis of

the amino-acids so liberated by the neoplastic tissue. Chisholm found that the disproportion is largely a matter of water content, as he found that in dry tumor tissue there was no constancy in this regard.

Investigations of the relations to cancer of single amino-acids serve to illustrate the inconstant character of unusual features of protein metabolism in this disease. Fränkl observed that while tryptophan was lacking in skin cancers and in melanotic tumors of the skin, it appeared in those conditions in excessive quantities in the urine. But that the abnormality of metabolism that this would indicate is variable, even with the special types of malignant growths considered by Fränkl, was indicated by Fasal, who found tryptophan in increased amounts in some skin cancers; he found it also in metastatic cancers of the liver, but it was lacking in a case of carcinoma of the breast studied by him.

The study of the peculiarities of the growth of malignant tissues *in vitro* has revealed a number of characteristics in which these differ from their normal prototypes in respect to nitrogenous metabolism. Carrel and Burrows reported in 1911 that the addition of the plasma of another tumor-bearing fowl to cultures of fowl sarcoma had the effect of a marked inhibition of growth, while the plasma of the tumor-bearing host was without this action. The addition of sarcoma extracts caused increased growth not only of the tumor cells, but of normal tissues as well, and surpassed in this respect extracts of embryonic tissues. A similar effect of apparent immunity was shown by the addition to cultures of mouse sarcoma of the plasma from tumor-bearing rats, while the plasma of normal rats permitted free growth, as shown by Lambert and Haynes, who ascribed the inhibitory action to cytotoxins. A familiar finding in work with tissue cultures was that embryonic extracts were almost essential for growth, while the plasma could be substituted by other substances, as for instance calcium in colloidal solution, as was shown by Drew; so apparently the unusual effects of what may be termed immune plasma would not appear to be associated with nutritive and thence metabolic factors. The same statement is also probably true of the inhibitory effects on tissue growth shown by the plasma of aged persons, as shown by Carrel, or by extracts of adult tissues, found by Mottram. On the other hand, the effect of embryonic extract, and also of the extracts of sarcomatous tissues, would appear to be due largely to nutritive elements contained in them, as was found by Baker and Carrel largely in connection with cultures of normal tissues. They found that, as a substitute for these extracts, partially digested proteins from various sources might be used; but while these proteoses sufficed, the substitution of amino-acids did not suffice, for the building up of new cellular protoplasm, although they had the effect of promoting cellular migration. In these respects there was not strict correspondence between normal and neoplastic tissues; with the

partially digested products of casein or egg albumin, the proteoses were suitable for the growth of normal fibroblasts, while for sarcomatous fibroblasts the further addition of glycocoll and of nucleic acid was necessary for rapid growth. On the other hand, partially digested material from liver permitted unlimited growth of sarcoma cells, while it served only temporarily for the growth of normal cells. That different tumors show variant needs in the way of nitrogenous material was shown by Carrel in connection with various tumor strains. Further differences between tumor and normal tissues were shown by Fischer. He found that with a strain of mouse carcinoma the addition of rat serum would suffice for nutriment in the absence of embryonic extract, as would such extract after inactivation at 56 C.—features lacking with normal tissues. A characteristic of malignant growth that Fischer particularly emphasized is the ability of tumor cells to obtain their nutriment from nearby cells with which they come into contact. Zakrewski, in a study of a number of different tumors, found that this ability to destroy and utilize normal cells is not a constant property of malignant cells, and that the liquefaction of the plasma clot that adds so greatly to the difficulties of culture of many malignant tissues also occurs in normal tissues in circumstances of cellular disintegration. It would seem that this feature is accomplished by a similar process in the case of neoplastic tissues, particularly as Zakrewski found the latter unusually sensitive to intoxication by products of cellular decomposition.

In regard to nucleic acid metabolism, the study of cancer has revealed as little in the way of specific changes as with nitrogen metabolism in general. Bang in 1904, as a result of determinations of nucleic acid content in the metastases of a malignant testicular tumor in lymph glands, found in them approximately the same content as that of the invaded tissue, and Beebe made a somewhat similar observation in that while he found nucleohistone absent as a rule in primary tumors, it was present in their metastases to lymph glands, from which he inferred an influence in this respect of surrounding tissues on the processes within the cancer cell. Tracy could find nothing peculiar in the phosphorus content of malignant tumors, but Beebe, in common with Petry and Wolff, found in malignant tumors a relatively high proportion of nucleoproteins. Probably largely from these findings Skerrett inferred that cancerous growth required an especial abundance of nuclear material, which he surmised was obtained from the disintegration of adjacent cells. In 1912 Calkins, Bullock and Rohdenburg reported that toxic substances, presumably belonging among the nucleoproteins, were capable of stimulating to excessive mitosis even relatively fixed tissues—pancreas and liver—with the appearance of changes which they were inclined to regard as precancerous. They advanced

the hypothesis that, as a result of local conditions which might be determined by a variety of causes, products of local autolysis were liberated, which stimulated the division energy of previously latent cells. This is essentially similar to the necrohormone theory as advanced by Ross and again and more recently in connection with postulated changes of more general character by Willy Meyer.

The finding by Ordway and Morris that there is a marked increase in the urinary uric acid output in cancer, might presumably be interpreted like other abnormalities of urinary elimination in cancer, as being brought about by excessive destruction of tissue. Wolter, in a comparison of the phosphorus contents of metastatic cancer of the liver and the adjoining hepatic tissue, found a diminished amount in the former, principally as a result of a reduction in the amounts of lipid and protein phosphorus; but the latter was increased as compared with normal, noncancerous liver. Ensleme found evidence of increase of nucleic phosphorus in malignant tumors, with a content ranging from 28 to 35 mg. per hundred grams of fresh tissue, as compared with from 13 to 21 mg. for corresponding normal organs. Goodman found the presence, in nonulcerating mammary cancers, of considerable quantities of nuclease, with the presence of which he associated the occurrence of cancerous cachexia.

A number of writers have asserted that in association with cancer there is marked alteration of the phosphorus content of the circulating blood. Von Moraczewski reported in 1895 that cancerous persons showed a diminished amount of blood phosphorus, but to no greater degree than occurs in anemia from any cause. In 1921 Gröbly found that in proportion to the number of red cells the phosphoric acid radicals in the blood are considerably increased, a finding that has been confirmed by Vorschütz and Vorschütz, by Zerner and by Wohlfarth, although the last named observer found a similar increase in cachexia from other causes as well. Buckman, Minot, Daland and Weld, however, found that the total phosphorus content of the blood is roughly proportional to the hemoglobin content, and like Wohlfarth are inclined to attach little special significance to this finding in cancer, a view which is shared also by Byrom and Kay.

#### NECROHORMONES

The idea that malignant growth might be the result of a discharge of growth-stimulating material from dying or degenerating cells has been advanced a number of times. In 1902 Marchand, in a comprehensive discussion of tumor etiology, after discounting the theory of parasitic origin, expressed the belief that in certain conditions of cellular degeneration there are formed toxic materials the accumulation of which lead to a change of cellular behavior, with a liberation of the affected

cells from normal regulatory mechanisms. In 1903 Clowes, although he favored the parasitic theory, acknowledged the possibility of an action of stimulant toxins formed within the cell or at a distance from it. Skerrett in 1907 surmised that the superabundance of nuclear material that he regarded as essential to cancerous growth might find its source in the disintegration of the nuclei of dying cells. In 1910 Stoeber and Wacker observed that on injection into the rabbit's ear of products of protein cleavage—pyridine, indol and skatol—growths were obtained closely resembling human skin cancer, and suggested that malignant tumors might owe their origin to an excess production of similar substances, and their anchoring by the proliferating cells. Bruntz and Spillman, in the following year, considered leukocytic infiltration as leading directly or indirectly to epithelial proliferation, and if this stimulation were sufficiently prolonged, the actively proliferating cells would divert more and more of their energy to this, and ultimately abandon their functional activities and become permanently committed to a process of unlimited growth. In 1911 and 1912 Ross reported observations in which he found that leukocytes, after exposure to extracts of dead and decomposing tissues, underwent rapid division. The substances responsible for this he termed auxetics, and found that, as a rule, they were such as contained the amidine radical. There appeared as well to be a second class of substances, which he termed augmentors, which had the property, as his name for them suggests, of augmenting this growth-stimulating action. He advanced the hypothesis that malignancy is due to the discharge of these substances from necrotic tissue, with a resultant stimulation of the regenerating tissues to unlimited growth. The process could become continuous as a result of the continuing death of some of the cells, serving as a constant supply of the stimulating materials. Rohdenburg and Bullock in 1912 considered that the continued presence of nucleoprotein hydrolysis, if removal failed to take place, could act as a stimulant to continued cellular reproduction, and Calkins, Bullock and Rohdenburg, as stated, found that substances of this sort could stimulate innumerable mitoses even in relatively fixed tissues, such as pancreas and liver, although definitely cancerous growth was not observed by them. Jones and Rous in 1914 found that injury of tissue appeared to be essential to the successful intraperitoneal implantation of mouse tumors; their explanation, however, was that in these circumstances there was greater certainty of provision of stroma for the introduced tumor. In 1916 Calkins announced that extracts of epithelial tissues in general had a growth-stimulating effect on free-living protozoa, but that cancerous tissues had an added lethal effect; he suggested that the stimulating substances might be of autolytic character, and that cancer might be due to their liberation and constant action in a focus of continuous destruction and repair of tissue. Robin in 1919 advanced

the hypothesis that cancerous activity might be the result of intense proteolytic activity around, not in, the malignant focus—the excess of water in and about malignant tissues being his evidence for such activity—and that resynthesis of material set free in this way could serve as the cause of the unrestricted growth. Caspari in 1922 regarded the necrotic portions of tumor tissue as a possible source of nutritive material for surviving tumor cells, and Haberland regarded such substances as definitely in the nature of growth-promoting hormones, which however might be in part of extraneous origin. That radiant energy stimulated excess tissue growth by means of the liberation of necrohormones from irradiated tissues was suggested by Caspari in 1923. In that same year, Drew described his experiments, previously cited here, in which he found that tumor extracts and extracts of autolyzed normal tissues had growth-promoting powers. Crawford in 1924 suggested that the scrotal cancers of cotton-spinners were due only indirectly to the lubricating shale oils, the accumulation of dead and dying epithelial cells in the scrotal folds being the element directly responsible. Eastwood in 1925 considered that cancer originates in circumstances of exclusion of the implicated cells from access of growth-regulating elements of the plasma, without nutritive deficiency, the stimulation to growth being furnished by the autolysis of cells that have succumbed. Essentially the same idea forms part of Willy Meyer's theory of the inception of tumor, as well as of that most recently expressed by Caspari, and Müller would see cancer as caused by necrohormones that are specific in that they are derived from disintegrated tissues of the sort which they stimulate to reproduction.

There can be little question that growth-stimulating materials occur in tumor tissue; Drew's experiments with tissue cultures; those of Erdmann and Haagen, and Burrows, in which the injection of tumor filtrates alone or in conjunction with embryonic tissues determined the inception of new tumors, even though possibly infrequently; those of Eggers, who could observe delimited overgrowth of regenerating connective tissue after similar injections—all indicate such stimulating action. As to the nature of the stimulating substances, they are usually regarded as products of protein cleavage; Wright found that similar substances obtained from embryonic tissues could pass through collodion filters impermeable to proteins, and Phillipson was of the opinion that complexes containing the pyrrole ring were responsible for stimulation of this type, not only as derived from disintegration of tissue, but as the responsible agent as well of most known cancerogenic chemical substances.

The principal objections to the several theories of necrohormones as the agents responsible for cancerous growth are based largely on indirect evidence. If such substances played the important part assigned to them

in some of the theories, it would not be unreasonable to expect cancer to occur more regularly in circumstances of continued destruction of tissue accompanied by regeneration, as for instance in lupus, which, while precancerous with some frequency, is not so at all regularly. Also, the limitation of malignant growth to cells of a single origin, which is so usual that exceptions are invariably considered deserving of special report, would appear incompatible with these theories, unless one accepts Müller's idea of specific necrohormones, which again is scarcely in accord with what would appear to be relatively simple chemical composition.

#### FAT AND LIPOID METABOLISM

With regard to the metabolism in tumors of fat in the strict sense of that word, there does not appear to be any constant or significant alteration. In 1906 Dunin-Karwicka, in the course of a general study of fat metabolism, found that vital synthesis of fat could occur only in living and nucleated cells, in circumstances which determined its deposition in droplet form, and established the presence of such fat in living tumor cells. Beatson in 1911, as a result of studies of tumor fat made by Duncan, in which he found a gradual increase in iodine value with advancing age, and an especially high value in cases of cancer, was inclined to attach special and possibly etiologic significance to the increase in unsaturated fatty acids, and while Dominici corroborated this work in the same year to the extent of finding fats present in all the tumors he studied, and finding them different from normal fats, Wacker, in the following year, was quite unable to confirm Duncan's work and the surmises of Beatson. Wacker found that the change in cancerous fat was one of increase of unsaponifiable material, including cholesterol—a change which was not specific for cancer, as it occurred as well in a number of wasting diseases. More recent work by Currie is in partial agreement with that of Duncan, since he found in 1922 that the fat of malignant tissues shows marked variations in iodine value, the local fat of carcinomas showing the greatest proportion of unsaturated fatty acids, while that of sarcomas and chronic inflammatory lesions had a value intermediate between those of normal tissues and carcinomas. In 1914 Bullock and Cramer reported that while variations from normal fats and lipoidal substances in general were frequent in the transplantable tumors of rats and mice, these differences were not constant, and involved even different strains of tumor cells from the same parent tissue. That fats could act as chronic irritants, and so possibly display cancerogenic action, was shown by Yutaka San in 1916 and 1917 and by Kon in 1917, who found that in rabbits and rats after long feeding with hydrous wool fat there was deposition of lipoidal material in the wall of the stomach, which was associated

with papillomatous or adenomatous overgrowth of the epithelium of the mucosa. Lee, Fukuda and Kinoshita in 1924 reported that the feeding of hydrous wool fat to tarred rabbits hastened the development of tumors and accelerated their growth after malignancy was initiated, while with mice Kashiwagi, Fukuda and Owaga found that the administration of hydrous wool fat so greatly shortened their lives as to reduce greatly the incidence of tumor.

A series of studies on the lipatic ferments of tumor tissue made by Noyes, Sugiura and Falk gave results that indicated marked similarity in the lipases of a number of rat tumors, but these differed much from the ferments of mouse tumors, and both were different from the lipases of the Rous sarcoma. As regards tumors of man, two main types of lipases were found, one with reactions resembling those of the lipases of the Flexner-Jobling rat carcinoma, the other quite different. Even in tissues of common derivation—uterine fibroids—two types of lipatic activity were found.

With the lipoids, as distinct from fats, findings of more significance have been obtained. Bossart in 1902 found that in undegenerated malignant tumors there was a considerable amount of lecithin, but little of true fat, the latter appearing in quantity in circumstances of degeneration and necrosis. Werner in 1905 was inclined to ascribe the effects of the x-rays and radium to their action on lecithin, in rendering this more susceptible to ferment cleavage. Reinke in 1908, from studies conducted on the brain of the larval salamander, in which he observed atypical growth after the action of ether, ascribed this to injury of intracellular lipoids, which he regarded as exercising a physicochemical restraint, interference with which occasioned unregulated growth. In similar experiments with embryonic tissues implanted in rats, he obtained some evidence of increased, but not malignant, growth, as was also the case when saponin was used instead of ether. Meyer in 1909 presented an elaboration of Reinke's idea; he regarded cellular structure as essentially an emulsion of colloidal proteins and lipoids of such nature as to prevent homogeneity and free chemical activity, with the lipoids forming a protective membrane on the cell surfaces. Other views implicating the lipoids in the genesis of malignant growth are those of McDowell, who considered them as active maintainers of anabolic activity, with malignancy the result of their excessive accumulation. Some experimental support for this conception was furnished by the work of Rosenheim, who found that cholesterol hindered to a great degree the acceleration of fat-splitting effects by bile salts and similarly acting substances, and by that of Wacker and Schminke, with their finding that in general such substances as cause epithelial hypertrophy are lipoidal or lipoid-soluble. Robertson and Burnett, and Sweet, Corson-White and Saxon, found in 1915 that



the administration of cholesterol to animals in which tumors had been implanted facilitated the growth of the tumors and their tendency toward metastasis, and Rondoni has recently reported that the injection of cholesterol greatly enhances the growth of tumors in mice, while that of lecithin restrained it. Luden in 1916 described certain associations between cholesterol metabolism and malignant growth. Not only did she allege a considerable increase of blood cholesterol in malignant disease—a feature that she associated with sex gland function, thereby linking together the increased incidence of cancer at the period of diminishing activity of these glands and cholesterol metabolism; but she postulated further effects in that excessive blood cholesterol was regarded as weakening the defensive mechanism of the lymphoid tissues and promoting cellular multiplication as well. Luden's report of excessive blood cholesterol in malignant conditions has received no very general confirmation. Roffo and Burgheim agreed with Luden, the former finding increase of cholesterol in the serum of patients with cancer, and even to a greater degree, in the tumor itself. Roffo also reported that he had tested for cholesterol content the tumor-afferent and the tumor-efferent blood in fowls, rats and man and found evidence of marked diminution during passage through the tumor, amounting to from 15 to 69 per cent—indications, he believed, of its utilization or destruction there. Denis, however, found that in almost every case studied by him the blood cholesterol was within, and sometimes even below, normal limits. Currie found it slightly deficient, and Mattick and Buchwald found that reduction of plasma cholesterol occurred as a rule. Jacobs reported that after irradiation there was an increase of free cholesterol within the tumor cells. Bolaffi found a pronounced increase of combined cholesterol in tumor-bearing mice, with particular concentration in the tumor itself; but he found little difference in the cholesterol content of the blood of normal and tumor-bearing animals.

In addition to the enhancement of tumor growth which has been reported to follow the administration of cholesterol, is that found by Robertson and Burnett to follow the administration of tethelin, a lipoidal substance isolated from the anterior portion of the pituitary gland. Erdmann found that the injection of this into embryonic tissues implanted in fowls caused increased, though still delimited, growth.

There are a number of experiments in which attempts were made to ascertain more or less directly the action of lipoidal substances on growing tissues. Borst reported that in a certain number of rabbits to the diet of which cholesterol was added, there was an enhancement of the local inflammatory reactions and the cancerogenic effects of the application of tar. Remond, Sendrail and LaSalle observed that while the cholesterol content of the blood of tarred rabbits is usually increased during the precancerous stages, there is usually an abrupt

fall with the onset of actual malignancy, which is followed by a gradual return to normal levels. Shimoda, on the other hand, found that growth of tar cancer is slow in rabbits with a normal blood cholesterol content, and that there is a definite relationship between cholesteremia and rapid growth of tumor. With tissue cultures, Baker and Carrel found that a large element in the growth-inhibiting effect of the serum of aged persons is due to its lipoidal content, with conditions of increase of total lipoids and lecithin and relative diminution of cholesterol.

Of the more recent theories implicating lipoids in the causation of malignant tumors, that of Sokoloff is essentially similar to the views of Reinke and Meyer, in that he regarded the primary change in the inception of malignancy as one of alteration of cellular membranes and of intracellular lipoids, with a change of lipoidal equilibrium that makes cancer not so much a disease of the cell as one of intracellular relationships. Crile regards as the fundamental feature of living tissues the accumulation of free energy on the dielectric lipoidal films that separate the nucleus, the cytoplasm and the intracellular spherules. According to his theory, cancer cells must have a large electrical storage capacity, with a conductivity different from that of normal cells. He stated that an actual test showed this high dielectric capacity in cancerous tissues—a finding that is in accord with that of Fricke and Morse—and a correspondingly high conductivity, these accounting for the abnormal growth energy of cancer. The experiments of Waterman, while they indicate a disturbance of electrical relationships within the cancer cell, do not bear out this conception of Crile. Waterman found that in attempts to measure the electrical resistance of living tissues by means of the Wheatstone bridge, using an alternating current and a telephone receiver, there can be found no definite minimal tonal point, owing to polarization at cell limits. This can be offset by suitable condensers, and by means of these it is possible to measure the potential of this polarization. If the relationship of polarization to resistance is expressed by the fraction  $P/W$ , in tumors this is very small, often in fact 0. If  $W$  is expressed in terms of its reciprocal, conductivity, according to Waterman's experiments  $P/W$ , which now becomes  $PC$ , is very small, while according to Crile's hypothesis, with both capacity and conductivity high, it should be large. Waterman explained his results as due to heightened permeability, which permits both anions and cations to migrate freely and equally—an alteration which, like Crile, he ascribes to lipoidal disturbance. In connection with the action of calcium salts on cancerous tissues, Waterman had found that the effect of these is to raise the ratio  $P/W$ . As a possible explanation of the altered lipoidal metabolism that appears to be rather definitely established in malignant tumors, Corran and

Lewis found that in cancer serum there is less of lipolytic co-ferment than in that of normal individuals. Rondoni, working on the theory that restriction of lipolysis would restrain proliferation in tumors, attempted to achieve this by the injection into tumor-bearing rabbits of lipoidal mixtures, but got at best limited restriction of growth of tar cancers.

The discovery announced by Freund and Kaminer in 1910, that the serum of noncancerous persons had the property of destroying cancer cells—a property that was lacking in that of sufferers from cancer—was confirmed by Neuberg in the following year and was ascribed by him to the presence in cancerous serum of antiferments, produced in response to the discharge into the circulation of ferments originating in the cancer; this was in accordance with the finding of Freund and Kaminer that cancerous serum could inhibit the lytic action of normal serum. Kraus, Graff and Ranzi found that a similar effect was manifested by placental serum, as well as by that of gravid women up to the tenth month after the inception of pregnancy, after which the lytic property gradually reappeared. While Freund and Kaminer were inclined to view the phenomenon as one associated with predisposition to cancer, these later workers, like Neuberg, considered it as due to metabolic alteration incident to the development of rapidly growing tissues. Subsequent finding by the original discoverers that the change was not constant (they reported its occurrence in 88 per cent of 113 cases of cancer), and that it disappeared for a time after the extirpation of the cancer, was in accord with this view, although in a later report Freund and Kaminer reversed their finding of reappearance of lysis after removal of the tumor. In subsequent publications, Freund and Kaminer established the fact that the process was scarcely to be regarded as one of ferment activity, but was due, they believed, to the presence of certain fatty acids, from the effects of which the malignant cells in the cancerous subject were protected by an abnormal nucleoglobulin. The presence of this they still regarded as one of the essential elements in predisposition to cancer. The dicarboxylic acids, which appeared to be the potent lytic agent, they considered as a product of normal intestinal digestion, and the neutralizing agent as the result of production there of unsaturated fatty acids, which, joined to nucleoglobulin and carbohydrates, formed complexes which had that effect. To chronic irritation they assigned the rôle of local exhaustion of the lytic acids, as well as the possible local accumulation of the neutralizing nucleoglobulins. The parallelism between cancer and embryonic tissues in this connection which had been noted by Kraus and his co-workers was confirmed by Rosenthal, who found marked similarity in the behavior of both carcinomatous and fetal serums and tissues, as did Kraus and Ishiwara. Hirschfeld made the significant observation, as concerns the relations of

the phenomenon to cancerous predisposition, that in animals with implanted tumors the action of the serum was the same as that of individuals with spontaneous tumors. On the other hand, as corroborating its significance in predisposition there are the findings of Nather and Orator, and of Peracchia, that the lytic property normally tends to disappear in persons at about the forty-fifth year of age, although it may be absent in younger persons as well; also the fact reported by Freund and Kaminer, that the lytic property for cancer cells of the skin may be destroyed by the application of irritants of cancerogenic type, as tobacco juice or pyridine, and the observation of Waterman and de Kromme that in the induction of tar cancers there is a final complete abolition of the serum lysis, preceded by a lessening of it during the precancerous stages. In addition to being a property of normal serum, the lytic power is shown by tissue extracts, as found by Kaminer and Morgenstern, and Waterman, de Kromme and Lemmens. The former observers found that it was possessed to an unusual degree by thymic extracts, although it was absent in the thymuses of cancerous individuals. Waterman and de Kromme found it in especial abundance in reticulo-endothelial tissues; they also found that its activity was limited to a narrow range of hydrogen ion concentration, and that it was sensitive to irradiation.

Attempts at identification of the agent yielded to Freund and Kaminer, as previously stated, the conception of it as a dicarboxylic acid, similar in many respects to maleic acid, but of considerably higher molecular weight. Goldzieher and Peterfi found that suberic and sebacic acids have a cytolytic action very similar to that of the agent in question. Waterman, de Kromme and Lemmens, on the other hand, were unable to identify it as an aliphatic acid, and regarded it as a combination of nucleoproteid and lipoidal material. Like Freund and Kaminer, they could find no relationships between its behavior and that of known ferments. The test has received some investigation from the point of view of possible utilization as a general and much needed diagnostic reaction. Although Koritschoner and Morgenstern found that it could be demonstrated by refractometric methods, it does not appear to be sufficiently constant to be reliable in the diagnosis of cancer. Coca found that the cytolytic action of normal serum is so dependent on uncontrollable factors as to make the test unreliable; Frankenthal found that the reaction is neither constant nor specific; Leitch found the test of no practical diagnostic value, and Herly was unable to find that normal serum applied to the Flexner-Jobling rat carcinoma had any deleterious effect on the transmissibility of that tumor. In a more recent publication, Waterman, de Kromme and Lemmens asserted their belief in the importance of the reaction in relation to predisposition to cancer; as concerns the character of the lytic agent, they considered it as akin to other

immune bodies such as occur to some extent spontaneously, and would place it among the lipoproteins.

#### CARBOHYDRATE METABOLISM

The earlier literature on cancer contains little reference to its relations to carbohydrate metabolism, although Rogers suggested in 1903 that this phase of metabolism might be connected with the cause of malignant growth, basing his hypothesis largely on the presence in tumors of glycogen, which had been reported by Brault in 1894 as occurring in them more or less proportionately to their rates of growth, and on the fact that Buxton had found in them quantities of amylase. The evidence for increased glycogen content was questioned in 1907 and 1908 by Haaland, who with mouse tumors could find no relationship between its presence and the rate of growth. Chisholm in 1910 and 1911 described a study of the respiratory exchange of mice in which tumors had been implanted; ordinarily there was little difference between these and normal mice, but after a carbohydrate meal there was a rise of respiratory quotient and in the excretion of carbon dioxide, which was slightly greater in the mice with tumors. A rather significant finding was reported by Cramer and Lochhead in 1913, to the effect that in tumor-bearing rats there was a more rapid exhaustion of hepatic glycogen, without, however, any indication of increased oxidative activity. They inferred that the carbohydrate was used for the synthesis of new protoplasmic material. Rhodenburg, Bernhard and Krehbiel announced in 1919 that they had found an unusual persistence of blood sugar in cancerous patients after the feeding of dextrose, with a characteristic tolerance curve which they believed might have a diagnostic value—a finding which has since been confirmed by Foerster and Foerster, who, however, also found this change in other forms of debility as well. DeNiord and Schreiner in the same year could find no characteristic changes in blood sugar nor in diastatic activity of the blood. In a later report, Rohdenburg and his colleagues stated that persons with cancer showed in larger proportion moderate reductions of blood sugar than did normal persons—a finding that was particularly likely to be the case with those showing hepatic and gastro-intestinal cancers. Lewis in 1922 observed that in cultures of fibroblasts the presence of dextrose was necessary if rapid degeneration was to be avoided, although excess of the sugar led to undue acidification of the medium.

In 1923 Warburg, in company with Minami, announced what may be surmised to be the most fundamental discovery in connection with the metabolism of cancer—the possession by carcinomatous tissue of the property of splitting dextrose into lactic acid to a degree at least 70 times that of normal tissues. An even greater effect was obtained if

respiratory activity was inhibited, as by narcotics or hydrocyanic acid, and the energy released in this process was comparable with that resulting from respiration. In later papers, Warburg, with Negelein, Posner and Wind, supplemented the original statement with the results of more detailed study. Even in an atmosphere of oxygen, the energy of malignant tumors is derived in part by glycolysis. A number of normal body tissues were found to share partially in the property, and embryonic tissues particularly, although with these there is almost complete cessation of permanent glycolysis in aerobic conditions. The only normal tissue that manifests the property of aerobic glycolysis is, rather curiously in view of its limited capacity for regeneration, that of the retina. In the process 1 molecule of dextrose yields 2 of lactic acid; with an ample supply of the former, this activity may be maintained indefinitely by the tumor, and after it has been stopped, as by freezing with liquid air, further growth appears to be impossible, as tested by transplantability. While malignant tumor tissues would appear to get the greater part of their available energy from this glycolysis, a certain amount of accompanying respiratory activity would appear to be essential, as Warburg found that the capacity of survival in strict anaerobiosis is limited to about twenty-four hours if sufficient dextrose is available; when both types of metabolism are interfered with, survival is for less than four hours. An especially interesting relation is shown by benign tumors, with which it was found that while they show the property of anaerobic glycolysis to a degree comparable with that of cancer and embryonic tissues, their power of permanent glycolytic cleavage in the presence of oxygen is relatively slight. A certain amount of aerobic glycolysis may occur in normal tissues in conditions of partial asphyxiation; but here it is accompanied, as is not the case with malignant tissues, by progressive degeneration and eventual death. That a certain amount of residual respiratory metabolism is necessary for the indefinite survival of tumor tissues has also been found by Wright.

A characteristic feature of this metabolic phenomenon is the discharge of lactic acid from the cells concerned, in which respect there is a marked difference from the more usual processes of lactic acid metabolism. Rostock in 1921 had found that the injection of lactic acid into mice in which tumors had been implanted caused a marked increase in the virulence of the tumors, and Tomita observed that in the developing chick embryo there was a rapid increase of this acid until the fifth day of development, after which it diminished, apparently from exhaustion of available dextrose. Bierich in 1923 noted that the infiltration of surrounding tissues by cancerous cells was preceded there by a peculiar swelling of the connective tissue fibrils, which he was able to simulate artificially by the application of lactic acid solutions. He regarded these changes as more or less essential precursors of the

infiltration by tumor cells. There are as well a number of more direct evidences of the production of lactic acid by tumors *in vivo*. Glaessner, after the injection of dextrose into rats in which carcinoma or chondroma had been implanted, observed the urinary elimination of lactic acid, although this did not occur in animals in which sarcoma had been engrafted. He later reported similarly positive findings in carcinomatous persons and in mice in which cancers had been implanted. Cori and Cori, who found that the free-sugar content of malignant mouse or rat tumors was less than that of the normal tissues of those animals, were able to raise it by an induced hyperglycemia, and in these circumstances observed an increase of lactic acid to 3 or 4 times its normal level. Also, by a method of diffusion from actually circulating blood, they were able to find increased lactic acid both in sarcomatous fowls and cancerous persons. Lasnitzki found that a sarcoma of the rat induced by the injection of tumefacient bacteria produced lactic acid to an average of 7.4 per cent of the dry tissue weight, per hour, while in the normal liver and spleen of this animal the production ranged from 0.3 to 3.5 per cent. Direct estimations of the lactic acid of the general blood in cases of cancer have failed to yield significant results. Valentin found that this was somewhat increased, as did Schumacher, but only in cases of pancreatic or hepatic involvement. Mendel, Engel and Goldschneider studied the relations of blood lactic acid in the normal individual, and found that this tends in the resting state to assume a minimal value, constant for the individual, which is temporarily increased by muscular activity. In cancer, Mendel and Baruch found no evidence of increase, even after the administration of large amounts of dextrose; but as the infusion of lactic acid itself into the circulation caused no perceptible increase, they were forced to conclude that it was destroyed with some readiness at a site as yet unknown, possibly the liver. Büttner, too, could find no definite increase of blood lactic acid in early cases of cancer, although in advanced cases it accumulated apparently when the liver became the site of metastasis. Wind and von Ottingen found similar evidence of the destruction of lactic acid in the fetus. Fahrig likewise found that in most of the cases of cancer studied by him increase of lactic acid in the blood was associated with circumstances of impairment of liver function. He pointed out that even with an observed increase, its accumulation would not necessarily be due solely to the peculiar metabolism of cancer, as other factors—deficiency of oxygen, muscular disease and hepatic impairment, all conditions that might occur secondarily in cancer—could also be concerned. More direct tests have shown, in the hands of Bierich, that the local lactic acid content increases rapidly after removal of the tumor from the body—a

phenomenon explicable by greater anaerobiosis and restriction of diffusion. Stahl and Warburg showed, in the case of a carcinoma of the human bladder, that the amount of glycolysis as measured by manometric methods was 9.9 per cent of the weight of the tumor, per hour, whereas measured by lactic acid production it was 10 per cent per hour. By means of determinations of lactic acid tolerance in rats treated by injection of sodium d-lactate, Cori and Cori estimated the rate of the production of lactic acid in transplanted tumors, and achieved results almost identical with some of those reported by Warburg as obtained by direct measurements on excised tissues—690 mg. per hour per hundred grams of tumor, as compared with Warburg's rate of 700 mg. per hour. The equivalence of dextrose utilization and lactic acid production as reported by Warburg has been confirmed further by Baker, Dickens and Gallimore. A limited amount of study of tissue cultures in this connection has given results which indicate that the same metabolic change may be detected there; Demuth and Meier found in such cultures that considerably more lactic acid is produced by rat sarcoma and mouse carcinoma than by fowl and rat fibroblasts and chicken iris epithelium.

That there are peculiar relations between malignant growth and general carbohydrate metabolism has been indicated from other sources. In 1923 Braunstein called attention to the fact that with the development of cancer in diabetic patients there is not infrequently a disappearance of the glycosuria, and that in spite of the destruction of pancreatic tissue incident to carcinoma of that organ, there is seldom the appearance of diabetes in this condition. Braunstein offered confirmation of Warburg's work, inasmuch as in relation with the phenomena just noted, he studied the glycolytic activity of cancerous tissue and found that it could in most cases destroy from 30 to 40 per cent of available dextrose in from 12 to 24 hours. In a later and somewhat more detailed report, he announced that incident to the rapid growth of cancer in diabetic persons there is a lowering of blood sugar, and that the presence of urinary sugar in such cases might at times recur after excision of the cancer. Other work on the relations of cancer to general carbohydrate metabolism, also with reference to diabetes, is that of Roffo and Correa, who found that cancer in diabetic persons tends to run an accelerated course.

Although Warburg's findings have been questioned by Bauer and Nyiri on the basis of work done by them on the formation of lactic acid in tumors, there is a rather considerable body of more or less directly corroborative evidence. Okamoto found that while sarcomas and carcinomas differ much in their ability to survive in conditions of anaerobiosis, the power of survival of the latter can be considerably increased if there is a supply of dextrose available. Mahnert, and



Murphy and Hawkins in general confirmed the work of Warburg, although they did not get as clearcut differentiation into the several metabolic types of glycolysis; rat placenta, for instance, showed the anomalous behavior of malignant tissues—a finding that had been confirmed by Loeser, with opposite findings by Fujita—while with the spontaneous tumors of mice the glycolysis was mostly similar to that of benign tumors. Rona and Deutsch, and Rosenthal and Lasnitzki, also confirmed Warburg's results, and reported that glycolysis by malignant tumors was greater in anaerobic than in aerobic circumstances, indicating that the peculiar behavior of malignant tumors constitutes, on the whole, a quantitative peculiarity—an opinion that has been shared by Fahrig, Pentemalli and Crabtree, the latter with reference to regenerating tissues. Louros reported results somewhat at variance with those of Rona and Deutsch, in that the same values were obtained with anaerobic as with aerobic glycolysis in 15 cases of cancer, while in 9 there was an actual reduction of glycolysis in anaerobic conditions. Baker found that leukocytes are capable of aerobic glycolysis, in which respect his results are at variance with those of Warburg and a number of other workers. Blanchetiere found, by a technic different from that of Warburg, that tumor tissues utilize dextrose, but in greater quantity than that necessary for the amount of lactic acid produced, from which he inferred other phases of dextrose metabolism as well as the one emphasized by Warburg, whose most striking findings, in Blanchetiere's opinion, were in part due to associated sepsis. Krontowski and Bronstein found that rapidly growing tissue cultures show energetic use of dextrose, and that this is particularly marked with those of carcinomatous origin. Rondoni found it possible to accelerate the development of the precancerous epithelial changes of tar painting in rabbits by the injection of dextrose. Crabtree, who, as stated, regarded the aerobic glycolysis of tumor tissue as less a qualitative than a quantitative change, found that the relations between respiration and glycolysis show complicated relations to the sites of implantation of malignant tumors, with, however, the single constant factor of a high rate of aerobic glycolysis. The possible objection to the significance of Warburg's work on the ground that it was done almost wholly with excised and so abnormally situated tissues has been met by the work of Tadenuma, Hotta and Homma, who determined the sugar content of tumor-afferent and tumor-efferent blood, with the finding of a decided reduction in the latter, especially in circumstances of induced hyperglycemia. Cori and Cori found that the induction of hyperglycemia in tumor-bearing animals results in the elevation of the lactic acid of the blood to 3 or 4 times its normal value.

Efforts to connect the peculiar carbohydrate metabolism of tumors with known elements in that metabolism have not yielded anything

in the way of definite success. As concerns the relations of insulin to tumor metabolism, Silberstein, Freud and Revesz found that animals with tar or implanted cancers are more susceptible to insulin intoxication, but this applies only to the earlier stages of tumor development, and with large tumors the reverse relation may be observed. Rather significantly, however, the injections of insulin were found to hinder the growth of implanted tumors, and if given in very large amounts, to greatly reduce the incidence of successful "takes." Piccaluga likewise observed retardation of engrafted tumors by the injection of insulin, and Münzer and Rupp got some, though rather indefinite, evidence of restraint of growth of tar cancers by its injection, an observation which Boivin was unable to confirm, while Barral reported the directly contrary result of reestablishment of growth of apparently quiescent implanted sarcoma in the rat after the injection of small doses. That its administration would presumably result in diversion of dextrose from the tumor to other sites would appear to be indicated by Borghi, who found a marked reduction of the glycogen of tumors after its injection. As to the presence of insulin in tumors, Cori found it in traces in extracts of both benign and malignant tumors; Cramer, Dickens and Dodds, on the other hand, could not detect it in malignant tumors, and suggested that its absence might be a factor in their peculiar metabolism. Roffo and Correa were at variance with both groups of workers just cited, in that they reported the presence in considerable amounts of an insulin-like substance in a spindle cell sarcoma of the rat, although in a later report they stated that in the Jensen rat sarcoma its presence could be demonstrated only by the use of enormous amounts of tumor material. In both benign and malignant tumors of men they found it in relatively large amounts.

With regard to glutathione, Hammett would ascribe the peculiar metabolism of tumors entirely to their possession of sulphydryl compounds capable of alternate combination with and liberation of hydrogen. He based his hypothesis largely on phenomena observed in growing tissues—the precipitation of lead in them, apparently as a sulphur compound, in regions of greatest growth, as observed in root tips; the inhibition of growth by lead, and the fact that he and Reimann found evidence leading them to believe that thiodextrose, a dextrose compound containing the SH radical, operates to stimulate wound healing, as acid extracts of root tips stimulated root growth. The action of trauma in stimulating repair he would ascribe to the liberation of such sulphydryl compounds, and the cancerogenic action of tar, he believed, is due to its possession of them. Baker found that by the addition of glutathione and hemoglobin to a mixture of casein digest, glyocoll and nucleic acid, a medium is provided in which

sarcomatous fibroblasts grow for a time as well as in embryonic extracts, and his explanation is that the glutathione and hemoglobin function in vitro by the regulation of oxidation-reduction reactions within the cell as well as in the medium. Findings similar to those of Hammett with regard to growing plant tissues have been reported by von Euler and Johanson, who prepared from yeasts substances which greatly accelerated fermentation by those organisms. Hammett's results and conclusions, however, are adversely criticized by Morgulis and Green, who were unable to find any effect of various sulphydryl compounds on regeneration in the polychaete worm, *Podarke obscura*. There is no decisive evidence that compounds of the type of glutathione are greatly increased in cancerous growths. Bierich and Kalle found an increase of cysteine to appear progressively in both normal and tumor tissues after excision, and in organs of animals with large tumors they found no considerable reduction of glutathione. In general there would appear to be approximately the same amount of glutathione in malignant tumors as in normal tissues. While Yaoi and Nakahara found that the Rous tumor was very poor in respect to this, LeCloux, Viviani and Firket found in transferable mouse tumors more of it than in the corresponding normal tissues, but even so less than is found in the liver or in embryonic tissues. In their opinion, a high glutathione content does not connote malignancy, but simply an excess of cellular metabolism. Kennaway and Hieger found that the content of glutathione varied with the amount of cellular material, and was independent of the normal or neoplastic character of the tissue. Bierich and Kalle, in addition to the indirect evidence cited here, found the sulphydryl contents of normal and malignant tissues approximately alike. Holmes, on the other hand, found that malignant tumors of the rat and of man contain abnormally small amounts of glutathione, and in rat tumors she found that added glutathione was very slightly reduced, while Voegtlin and Thompson found in transferable mammalian tumors a high glutathione content, comparable with that of the liver, one of the organs normally richest in that substance. As regards the relations between reduced and oxidized glutathione, Voegtlin and Thompson found a higher than normal ratio of GS-SG to GSH in tumors, while Bierich and Kalle found that while as a rule GS-SG is greater than GSH, this relationship is reversed in cancer, as it is in liver, muscle and lens substance.

In connection with the relation of glutathione to cancerous predisposition, Voegtlin and Thompson found that the total content of this in the body diminishes with age, a fact that was observed also by Murray during the embryonic life stages of the chick. In view of the fact, however, that Hammett considered the relation of malignant growth to the sulphydryl compounds as one of increased sensitivity to them in

the tissues concerned, rather than as a response to quantitative increase, neither this nor the failure to demonstrate in tumors excessive amounts of glutathione are necessarily decisive arguments against his hypothesis. A theory apparently almost the opposite of that of Hammett is that of Grumme, who would seek the cause of cancer in sulphur impoverishment, on the grounds of elimination of tar and arsenic as sulphur compounds, high elimination of sulphur in cancer cases as reported by Salomon and Saxl, etc.

Other views of the nature of the unusual behavior of tumors with respect to carbohydrate were suggested by Waterman, who in 1924 reported that with tumor extracts he was able to enhance the glycolytic activity of normal tissues; apparently, from the results of Dische and Laszlo, this effect may be manifested within the tumor host, as they found that the hepatic tissue of carcinomatous mice showed increase of glycolytic power, although the kidney, the organ with which Waterman obtained his results, did not. According to Aldus, Chiari and Laszlo, the glycolytic agent of mouse carcinoma is carried in the filtrate, and is absent in the liver of those animals, and Landegger and Pirker found in the serum of cancerous patients, as well as in that of pregnant women and in that of some normal persons, a fermentation-accelerating substance which they regarded as essential for the development of cancer. Kraut and Bumm, on the other hand, while finding no increase in the glycolytic power of tumor filtrates, found, like Waterman, that these have the power of greatly enhancing glycolysis by normal tissues, having an effect in this respect of co-ferment rather than of ferment activity. Embryonic extracts were found by them to stand midway between normal tissues and malignant tumors in this regard, and the glycolytic activity of tissues appeared to be proportional to their possession of this co-ferment. Rosenthal found that a similar co-fermentative action may be developed by normal tissues when kept *in vitro* in aerobic conditions, as was indicated by their subsequently increased rate of aerobic glycolysis—a phenomenon that tumors failed to show with respect to dextrose, although it occurred in them with relation to fructose. Neuberg, Kobel and Laser were able to obtain from rat sarcoma by extraction with alcohol and ether or with acetone an active glycolase, but the co-ferment was destroyed during the process, so that while the ferment acted on hexose-phosphate, the methylglyoxal formed was not further changed, indicating the importance of the co-ferment in the later stages of carbohydrate cleavage only. As Lasnitzki pointed out, the aerobic glycolysis of tumor cells may be simulated by dying cells, but with the difference that in the one circumstance reproduction precedes death. He pictured the alteration of metabolism as a gradual one, of a similar character to what has been observed in partially asphyxiated embryonic chicks, in which monstrous

development follows from arrest of developmental processes to a greater degree than those of growth, but with the outstanding difference that in the case of malignant growth the alteration is irreversible. Warburg's explanation of the change that he discovered is on the whole the simplest; he pictured malignant tumor cells as formed of descendants of exceptional cells with unusually retained powers of the glycolysis normally possessed during the embryonic state, in circumstances in which they are forced to rely more and more entirely on glycolysis as a source of energy, and in which their more normal fellows perish. Still another view of the nature of tumor glycolysis was advanced by Bierich, who expressed the opinion that the accumulation of lactic acid in and about malignant tumors is essentially due to inability of the tumor tissues to resynthesize the lactic acid into dextrose in a manner similar to what occurs in more normal sites of lactic acid production.

#### PHYSICAL CHEMISTRY

In addition to the electrical phenomena reported by Waterman, changes in surface tension, permeability, etc., have been given a considerable amount of study in relation to cancer. That alterations in the character of surface contacts of cells would produce changes in their interrelations and modes of growth was shown by Loeb in 1902, in a series of experiments in which he transplanted epithelial cells into a colloidal medium (agar or blood plasma) within the animal. He observed changes suggestive of carcinomatous development, particularly in the way of abnormal cellular arrangements—incidentally in these experiments taking the first step toward the independent culturing of tissues. In 1914 Lambert observed that in tissue cultures the dilution of the plasma with isotonic salt solution had the effect of promoting more extensive cellular migration, without, however, showing any effect on the rate of cellular multiplication. In regard to intracellular fluid content, Cramer found that in both normal and cancerous tissues this was increased in circumstances of growth and was more or less proportional to the rate of multiplication; the increase he did not ascribe to increased osmotic tension, but to differences in imbibition. Larson, Cantwell and Hartzell showed that the growth of bacteria in nutrient broth is greatly affected by the surface tension of the medium, even to the extent that with some anaerobes growth could occur in aerobic conditions in mediums of reduced surface tension, though too great a reduction acted adversely. A somewhat similar effect of freer growth was observed with cultured normal fibroblasts by Hogue, in that these showed an enhancement of growth rate in hypotonic salt solution, along with a shorter period of cell survival.

A number of workers have reported a lowering of surface tension of the blood or serum of cancerous subjects—Roffo and Kopaczewski, Svehla, Bauer, Solowiew and Lederer, the latter two finding a similar though less pronounced variation during pregnancy as well. Svehla would associate the reduction of surface tension with a lowering of blood calcium content, as he believed he could establish direct relationships between the two. Sauer, on the other hand, was unable to find any essential differences between the surface tensions of serums of cancerous and noncancerous gynecologic patients. As regards cancer tissues, Kagan reported that their extracts show as a rule reduction of surface tension, as did Solowiew. Bauer approached the problem indirectly, with a study of the effect of reduced surface tensions on dividing cells, using for these fertilized eggs of *Ascaris*, and reported that with them the diminution of surface tension effects a marked promotion of cellular reproduction. Bauer, indeed, believed that the local lowering of surface tension is the fundamental factor in the causation of cancer. His theory is based on the following postulates: Cancerogenic action must operate on the surroundings of the cells concerned; it must tend toward their isolation; it must promote cellular division; it must be possessed in common by all known cancerogenic agents, and it must be universally present in cancerous growth—demands all of which he asserted are met by reduction of surface tension. He extended his theory to embrace even the metastatic colonization of malignant tumors, as he found that the expressed juice of spleen has a relatively high surface tension, that of muscle tissue an intermediate value, and those of lymph gland, liver and kidney relatively low values. Krontowski, Bereschanski and Majeroski investigated alterations of surface tension in tissue cultures, and found that in the medium there was no appreciable reduction during tissue growth, with the exception of a mouse carcinoma in which this occurred to a slight degree. However, in circumstances of aseptic cellular disintegration a marked reduction took place, and they suggested that this may be the mechanism that acts in regeneration, to say nothing of malignant growth. Kagan found that reduction of surface tension by the treatment of cancer tissue with tributyrin greatly enhanced its growth after implantation, and Katzenstein and Knake reported that substances that lower surface tension have the effect, in tissue cultures, of stimulation of epithelial, and suppression of connective tissue, growth; certain tissues, however, as liver, behaved exceptionally. Einaudi's finding that neoplastic cells in general have a greater volume than that of the parent cells, and a particularly greater nucleocytoplasmic ratio, would apparently fit in with the conception of lowered surface tension as a major factor.

To the extent that the presence of calcium salts operates to restrain growth, a possible explanation is offered by the finding by Magath

that after-treatment with isotonic calcium chloride solution the inhibitory power of tumor cells is greatly reduced. Such results are strikingly analogous to those obtained with collodion membranes by Brinkman and Szent-György. They found that treatment of these with alkaline salts of fatty acids rendered them permeable to dissolved hemoglobin, an effect that was irreversibly abolished by calcium chloride. Mendelieff reported that normal cells, after disturbance in a number of ways—by the application of tar or tryptoflavine or by exposure to the x-rays—show a series of changes in permeability—an initial increase, followed by diminution even below normal, and a final return to the normal or a hypernormal condition—while cancer cells do not show these variations and maintain a constantly high permeability. Magath reported similar findings. Lange and Henning, in a study of cellular elimination of phosphoric acid, found that the output of this was greatly enhanced in conditions of anoxymbiosis, and that in tumors there was, on the basis of such elimination as evidence, a regular increase of cellular permeability. Magath called attention to the fact that the effects of the x-rays on cellular permeability are paralleled by the known imbibition phenomena of cancer and by those of electrometric polarization as measured by Waterman, and that all indicate the association of cancer with alterations of cellular permeability. The similarities between the “forcing” of plants and cancerous growth, to which attention has been called by Boreschi, would appear to admit of explanation along the lines of alterations in permeability, but of impairment rather than augmentation of this. In both there is inhibition or limitation of respiration, along with an accumulation of acid products. Niethammer, working with a new forcing agent, mercurio-phenol-mercury, found that this, like the excitants of animal cell proliferation, does not penetrate into the cells, but inhibits respiration by surface action; this in turn is associated with the intracellular accumulation of acetaldehyde, hydrocyanic acid, reducing sugars and amino-acids, with a cumulative effect of elevation of total acidity—all changes that he associated with impairment rather than facilitation of intercellular exchange.

Recently Kostoff suggested that the cause of cancer is to be sought in physicochemical changes in the cytoplasm which have the effect of modifying cellular behavior without affecting reproductive function. As an illustration, he adduced the production of tumors in hybrid tobacco plants by the injection of extracts of one of the parent plants, in cases in which the two parents showed reciprocal power of protein precipitation.

#### GENERAL SUMMARY

It is altogether obvious that in spite of the tremendous volume of work that has been done in connection with cancer and its inception,

there is as yet no theory of its origin which is adequate to meet all the known facts, and which is supported by sufficient direct evidence to render it satisfactorily probable. This is not to admit, however, that the work already accomplished has been fruitless. To a certain extent, its value lies in its negative character; as a result of it, one may conclude with some certainty that there are some things which, from the etiologic standpoint, cancer cannot be.

First, it may be considered as definitely established that cancer is not infectious in the sense that its origin is due to the attack of a specific parasite. It is of course well proved that certain infections may cause malignant growth, but these are so diverse in character that to explain their effects on the common ground of parasitism would necessitate viewing them as carriers of a specific virus, rather than as causative agents in themselves. It is quite true that this view has been advocated, most notably by Borrel, and the peculiar experience of Blumenthal with certain of his tumefacient organisms would lend it support. But the production by A. Fischer of a malignant tumor with fibroblastic cells cultured *in vitro*, by purely chemical agencies—supported as this experiment is by the somewhat similar, but from the present point of view, less decisive, experiments principally of Carrel—would appear to exclude definitely this hypothesis from serious consideration. Even the Rous filtrable virus, which most nearly meets the conception of an independent causative agent, would appear from this experiment of Fischer to be of metabolic rather than parasitic character, even if the probability of this had not been recognized from the extreme specificity of the several viruses of this type. If more evidence is needed of the essentially nonparasitic origin of cancer, it is afforded by the work done on production of cancer by the various irritants. True these, too, might be explained as acting through a local reduction of resistance to a conjectured parasite. But the certainty with which some of them induce cancer in suited subjects; the practically complete nonsusceptibility of other animals even though closely akin phylogenetically to those that are susceptible, and as subject as they to malignant tumors in different circumstances; the character of some of the irritants themselves, particularly those involving radiant energy, along with the fact that in suitable animals, as the rat, the effect of the latter agencies is ordinarily on deep rather than on superficial tissues—these, in connection with the experiment of Fischer already cited, would lead to the almost certain conclusion that the effect of all these agencies is a direct one, and that the known cancer-inducing parasites are simply special types of cancerogenic irritants.

With reference to the older theories of the induction of cancer, the more recent experimental work has thrown little light on the validity of the so-called Cohnheim's hypothesis, and has even to a limited extent



given it some support. Experimentally, malignant tumors have been produced on the whole more readily with embryonic than with adult tissues. To the extent that this theory may be linked with modern information of cancer metabolism, as has been done by Warburg, it is impossible to say that it may not meet the facts adequately. The evidence against this theory is largely indirect. In view of the localized action at the point of application of most of the cancerogenic irritants, to explain their effect on these grounds would involve a conception of almost ubiquitous embryonic displacement, or more properly, perhaps, of persistence of cells of embryonic potentialities. Just as altogether too little is known of metabolic phenomena in individual cells to be able to say with any certainty that none of them differs in metabolic potentialities from its fellows to the extent demanded by Warburg's hypothesis, so, too, present ignorance does not permit one to assume this as an established fact. What is essentially a modern adaptation of Cohnheim's hypothesis, then, at present is and seems likely to remain a possible but not directly provable speculation.

In this connection, a theory recently advanced by Simons may be of some interest, even though it is quite largely of speculative character. He regards cancer as resulting from the stimulation of persistent embryonic cells in response to bodily need for a growth principle elaborated in them. It is somewhat reminiscent of one advanced by Ishihara, who believed that all the phenomena of cancer may be explained by a deficiency in a hormone found in umbilical jelly and placental tissue; but it is supported by a modicum of experimental evidence, since Simons found that in his experience wound healing was accelerated by insertion of embryonic tissues.

As to the theories that derive the cancer cell from the results of nuclear fusion, with, as offspring, cells of abnormal potentialities, there does not appear to be any foundation for them in the facts revealed by experimental, as distinct from morphologic, investigation. Since it has been possible to produce malignant tumors in vitro in circumstances that would appear to exclude absolutely the possibility of such fusion, these theories may be dismissed as vain speculations. The same cannot be said of von Hanseemann's theory of anaplasia, which nothing in the field of experimental study especially contradicts; however, as has been emphasized repeatedly, the theory is one of mechanism rather than of cause.

Regarding theories such as that of Ribbert, which would ascribe malignant growth to the abnormal relations incident to cellular displacement, nothing has been found in experimental study to support them, and here the fact that unusual agencies are necessary to evoke cancerous growth in misplaced cells, as in tissue culture or within the

organism, or even in implanted embryonic cells, would seem to relegate cellular displacement to a place of relatively little importance.

It would appear to be necessary to explain cancer as the result of metabolic anomaly, by which the cells involved achieve the property of unlimited and unrestricted growth as the result of perversion of their normal functional and nutritive relations. This view has been suggested frequently in the many theoretical speculations on the origin of cancer. It was advanced as long ago as 1886 by Steven, and since, in somewhat variant forms, by numerous others. In part it has been represented as a diversion of the normal expenditures of energy of the cell from its miscellaneous functions to the one demand of reproduction. Morphologic studies of most adenocarcinomas would give ground for such a view, but in circumstances in which one can evaluate more accurately the persistence of functional activities on the part of cancer cells, as in those of the endocrine glands, there would appear to be a frequent retention of special function in spite of the excessive expenditure of energy in cellular reproduction. It is much more probable that the energy change is not one of diversion, but rather of additional source, as suggested by the work of Warburg. To this enhancement of cellular reproductive activity a large number of factors appear to contribute, either solely or in summation—inherent growth energy, as of embryonic tissues, in which, as pointed out by Warburg and his associates, there is a rather considerable resemblance to cancer cells in their capacity to obtain energy by glycolysis; achieved growth energy, as from chronic regenerative changes, in which the same exceptional source of energy occurs; hereditary predisposition; secretory or endocrine imbalance. To these must be added an element or elements of as yet unknown character, since in the matter of chronic regenerative changes alone, those induced by certain, "cancerogenic" agencies, are effective with some regularity, while others result in cancer only rarely, and then apparently in the presence of other concomitant factors.

Concerning the mechanism by which this metabolic aberration is brought about, there are much speculation and little knowledge. It has been ascribed to the action of necrohormones—growth-stimulating substances liberated from dead and dying cells. It has been attributed to hormonal imbalance, by Shirlaw, Fichera, Walz, Haberland, and in part by Willy Meyer, and others. While, as has been shown by Loeb, hormonal disturbances do contribute to the occurrence of cancers in laboratory animals, they would appear to be only one of the numerous factors concerned in their causation, and there is little definite evidence in support of the view that they are of primary importance. The same observation is true of the attempts to explain cancer solely on the grounds of vitamin abnormality. Burrows and Jorstad would assign

the regulation of cellular reproduction to the interaction of a growth-restraining principle, which they regard as more or less identical with vitamin A, and a stimulant agency regarded as homologous with vitamin B. To the extent that the experiments of Saiki and Fujimaki and Erdmann and Haagen in the induction of cancers by imbalance of these vitamins indicate their importance in the causation of cancer, the comparatively low incidence of positive results would seem to denote that their effect, like that of so many other factors, is only a contributory one; nor is there any indication in the results of Drummond that there is any appreciable quantity of vitamin B either in tumors or in actively growing normal tissues.

It would seem that there is more knowledge of the nature of the aberration than of its mechanism. From the work of Warburg it is rather definitely established that abnormally excessive carbohydrate cleavage is the source of the extra supply of energy necessary for the excessive growth. But along with this there appear to be associated other factors—lowered surface tension, increased cellular permeability and a series of phenomena apparently related to this, namely abnormal lipoidal relations, electrical reactions and response to calcium salts, as well as added intracellular water content. Recent work would indicate an abnormal, or abnormally excessive, co-ferment as the primary factor in this special cleavage of dextrose, and to what extent this may be dependent on these other changes, and which of the several associated alterations is to be regarded as the primary one, with the others ensuing in sequence to it—of all this one is in complete ignorance.<sup>1</sup>

The problem is not necessarily unsolvable. In view of the developments of less than the last decade, it does not seem vain to hope that further experimental work will eventually correlate into a logical and connected whole the several peculiarities that characterize the behavior of the cancer cell, and furnish a clear and well established conception of the nature and origin of malignant growth.<sup>2</sup>

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1. In a rather recent summary of his views, Auler expressed the belief that cancer may result from a number of different mechanisms. Among these he included: embryonization of cells; cellular isolation, which may result from anomaly, trauma, chemical irritation, senility, chronic inflammation, hormonal abnormality, etc.; factors of individual predisposition, with, in his opinion, contributory to this an element of intestinal abnormality that results in intermediate products of polysaccharid digestion which in turn interfere with the cleavage of the monosaccharids; other elements, such as increased alkalinity of the blood, a relative increase of potassium salts and an altered content of fibrinogen and globulin in the blood, and anaphylactic disturbances affecting the nerve and hormonal supply to the part involved.

2. To save space, the bibliography has been omitted. The author will be glad to furnish any references to the literature cited in his review on the etiology of cancer that the reader might desire. It is contemplated that the review will appear in monograph form, and in this case, the list of references will be included.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Frederick E. Becker, assistant professor of pathology in the school of medicine of the University of Colorado, has died at the age of 43.

Roscoe R. Hyde has been appointed professor of immunology in the School of Hygiene and Public Health of the Johns Hopkins University to succeed the late Carroll G. Bull.

J. Florey has been appointed professor of pathology, and J. W. Edington professor of bacteriology, in the University of Sheffield, England.

**Phillips Prize.**—The American College of Physicians has awarded the first Phillips Prize of \$1,500 to Oswald T. Avery of the Rockefeller Institute for Medical Research for his work on the pneumococcus.

**Provision for Surgical Research.**—One million dollars has been bequeathed to the Boston City Hospital by the will of the late Charles H. Tyler for the establishment and maintenance of a laboratory for surgical research to be known as the George E. Sears Laboratory.

**Society News.**—The next annual meeting of the American Association of Pathologists and Bacteriologists will be held in Philadelphia on April 28 and 29, 1932. One half-day will be given to the discussion of tuberculosis and one half-day to a joint meeting with the American Association of Immunologists. The meeting will be in conjunction with that of the Federation of American Societies for Experimental Biology.

# Abstracts from Current Literature

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## Pathologic Anatomy

THE CEREBRAL LESIONS IN PURULENT MENINGITIS. FREDERIC WERTHAM, Arch. Neurol. & Psychiat. **26**:549, 1931.

In studying the cerebral changes in twenty-four cases of purulent meningitis, Wertham centered his attention on pale areas (clearings) in the cortex and white substance. In these areas many ganglion cells either dropped out or appeared shrunken and homogeneous; their nuclei were pyknotic, while other cells showed severe changes. The glia in the pale areas exhibited either regressive or progressive changes, depending on the location of the lesion. In the cortex, the pale areas (the clearings) were small, affecting one or more layers. The glia seemed to have been increased and appeared as clusters. The nerve fibers were tortuous and swollen, and at the junction of the white matter and cortex they were demyelinated. The olives appeared especially vulnerable, whole portions of which dropped out, and many ganglion cells disappeared. Of interest also were the ventricular changes, in the form of a wall-like formation of the ependymal layer, cufflike infiltrations of the blood vessels and subependymal proliferation of the glia. The blood vessels showed changes in both the meninges and the parenchyma—perivascular infiltrations and some splitting of the elastic membrane. The vascular changes as well as toxic substances that may affect the blood vessels and the parenchyma alike are held by Wertham responsible for the pale areas that he believes may account for the cerebral manifestations in patients who recover from purulent meningitis.

GEORGE B. HASSIN.

CONTINUOUS OBSERVATIONS ON LIVING GRANULATION TISSUE. J. TANNENBERG. Verhandl. d. deutsch. path. Gesellsch. **26**:145, 1931.

By a modification of the method of Clark-Sanderson, a celluloid chamber was inserted into the rabbit's ear, and therein the development of vascular granulation tissue was studied over a period of from six to eight weeks. The new method permits the use of high magnifications, even of oil immersion and the making of motion pictures. A photomicrographic film is presented, which shows all stages from the new formation of blood vessels to dense connective tissue. By application of moderate heat and light, the emigration of leukocytes from the blood vessels was demonstrated.

C. ALEXANDER HELLWIG.

THE BLOOD MONOCYTES IN INFLAMMATORY EXUDATE AND IN GRANULATION TISSUE. W. BUENGELER and B. FISCHER-WASELS, Verhandl. d. deutsch. path. Gesellsch. **26**:148, 1931.

India ink and other colloidal solutions were injected into rabbits for a period of from two to four weeks. Then, by intravenous injection of protein, monocytosis was produced. Venous blood was withdrawn from different organs: aural vein, femoral vein, inferior vena cava, renal, portal and hepatic veins, splenic vein and right and left ventricles. Analysis of the blood showed the greatest number of monocytes present in the liver and spleen. The hepatic vein contained from forty to fifty times more monocytes than the vein of the ear. There was the greatest difference in the number of monocytes between the right and the left ventricle, a fact indicating that many monocytes originating from the spleen and liver are retained in the capillaries of the lungs. Many of the monocytes were stained with india ink and were therefore regarded as reticulo-endothelial cells.

In rabbits which were prepared in the same way as in the first experiment, granulation tissue was produced. Many monocytes were found that were stained with india ink. These cells behaved, furthermore, exactly like true blood monocytes when supravital stain was applied (janus green and neutral red). The authors conclude from their experiments that monocytes of the blood and in granulation tissue are identical; i. e., they are reticulo-endothelial cells.

C. ALEXANDER HELLWIG.

THE PATHOLOGIC SIGNIFICANCE OF RESIDUE STRUCTURES IN MICROSCOPIC SECTIONS. W. GERLACH, *Verhandl. d. deutsch. path. Gesellsch.* **26**:163, 1931.

Systematic examinations were made of residue structures in microscopic sections of heart, lung, liver, spleen, thyroid gland, muscle, aorta and testis in 80 cases in which autopsy was performed. The results were not satisfactory. There are cases poor, as well as cases rich, in residues. The amount of residue increases with the age of the patient, but there are also young patients with large amounts of residues. The findings are inconstant in cases of tuberculosis and carcinoma. The chemical analysis is incomplete except for calcium, iron and perhaps potassium. The author therefore prefers the spectrographic method devised by Gerlach-Muenchen by which a photographic spectrogram is obtained from fresh material evaporated in the high frequency spark. In a case of silicosis, sections of the lung showed the presence of silicon, aluminum, calcium, magnesium, iron, copper and silver. In a minute specimen of gingival mucosa in a case of lead poisoning, lead was plainly detected by this method. In gallstones, copper was demonstrated. The distribution of copper and manganese in cirrhotic liver was shown to be very irregular.

C. ALEXANDER HELLWIG.

EXPERIMENTAL STUDIES ON THE EXCRETION OF URIC ACID. A. SCHULTZ, *Verhandl. d. deutsch. path. Gesellsch.* **26**:174, 1931.

Saturated colloidal solutions of sodium and lithium urate were injected intravenously into rabbits. After ten and thirty minutes, respectively, the left and right kidneys were removed and fixed in trinitrophenol alcohol. Paraffin sections were stained with methylene blue, by which the uric acid deposits stain a brilliant green. Within a few minutes after injection, abundant deposits of uric acid were found in the following portions of the kidney: (1) the capsules of Bowman; (2) the epithelial cells of the uriniferous tubules, especially the straight tubules, and the ascending parts of Henle's loops; (3) the lumen of the whole loop of Henle and the excretory tubes. It is the first time that the excretion of uric acid by the glomeruli has been demonstrated.

C. ALEXANDER HELLWIG.

THE COPPER CONTENT OF THE CIRRHOTIC LIVER. E. VON ZALKA, *Verhandl. d. deutsch. path. Gesellsch.* **26**:180, 1931.

Nineteen livers without evidence of cirrhosis contained from 0 to 8 mg. of copper per thousand grams. The copper content in thirty-four cirrhotic livers was determined. The values varied between 1.87 and 95.25 mg. (average 31.59 mg.) per thousand grams. In fourteen cirrhotic livers, or 41 per cent, the values were normal, namely, below 10 mg. In five of thirteen livers presenting Laënnec's atrophic cirrhosis, the amount of copper was increased, while four of seven livers showing Laënnec's hypertrophic cirrhosis had a higher copper content. A liver showing tuberculous cirrhosis showed a content of 24.4 mg.; another showing syphilitic nodular cirrhosis, 52.08 mg.

The facts that the copper content of the liver is not increased in every case of cirrhosis of the liver, and that, on the other hand, a large amount of copper may be found also in biliary, tuberculous and syphilitic cirrhosis, are not in favor of the theory that copper is an important factor in the etiology of cirrhosis. It is more likely that the high copper content is due to the retention of the metal

in the diseased organ. It is assumed that there is some relationship between the copper content of the liver and the function of the reticulo-endothelial cells in the diseased liver.

C. ALEXANDER HELLWIG.

IDIOPATHIC CYSTIC MEDIAL NECROSIS OF THE AORTA. J. ERDHEIM, Virchows Arch. f. path. Anat. **276**:187, 1930.

Erdheim believes that many of the reported cases of so-called spontaneous rupture of the aorta may have been due to the lesion described by him. The principal changes, which are at first limited sharply to the media, are necrosis and mucoid degeneration. The process may lead to the formation of relatively large mucoid cysts. Regenerative changes become associated with the degenerative ones, but there is no cellular exudation or infiltration. The ascending portion of the aorta is chiefly involved, but necrotic mucoid patches have been encountered throughout the entire course of the aorta. All the elements of the media, muscle, elastic tissue and connective tissue take part in the reparative process, the muscle being most active. Regenerated areas of the media are easily recognized by the preponderance of muscle and by the abnormal shape and course of the muscle fibrils. The regenerated tissue is not a physiologically adequate one and readily undergoes degeneration in its turn. In the regenerated areas, elastic fibrils are relatively few and small and stain faintly. The connective tissue is relatively somewhat diminished in amount. The etiology of the process is doubtful. Endogenous and exogenous intoxications, that with nicotine being included among the latter, have been considered causative factors. Experimental work suggests a possible relation to avitaminosis. Hyperadrenalinemia seems to be an important factor.

W. SAPHIR.

REORGANIZATION IN THE WALLS OF CALCIFIED ARTERIES. G. B. GRUBER, Virchows Arch. f. path. Anat. **275**:54, 1930.

Gruber presents a histologic study of five examples of ossification of calcified arteries. He limits the term organization, as used in his title, to the replacement of dead tissue by living tissue that participates in the general metabolism of the body, and leaves out of question any physiologic and functional regeneration of the vessel. The specific matters that engage his attention are the question whether fracture of calcified arterial plaques occurs during life, the mechanism of removal and absorption of calcium from the arteriosclerotic plaques, the process of bone formation in such areas, the formation of the cartilage occasionally seen, the reaction of calcium resorption to ossification and the question whether the ossification is to be considered a new stage in the arteriosclerotic process. That fracture of calcified plaques occurs during life is proved by the presence between the ends of the fragments of proliferating mesenchymal tissue, which has been likened to the callus of fractured bone. Proliferating mesenchymal granulation tissue, which is formed between calcium fragments, and which may invade unfractured calcified material from the walls of the vasa vasorum, has the property of removing calcium by absorption and by phagocytosis. A syncytial foreign body giant cell tissue may be formed. The mesenchymal tissue may differentiate in several different directions. It may form fibrillated connective tissue, adipose tissue or marrow. From it may be developed osteoblasts that form bone. Gruber believes that bone formation in calcified arteries occurs through osteoblasts and rarely, if ever, by direct ossification of fibrous tissue. Cartilage likewise is formed by differentiation of the mesenchymal tissue. The cartilage may reveal evidences of both resorption and transformation into bone. For the resorption of calcium and for the fragmentation and removal of dead calcified material, proliferating mesenchymal tissue is necessary. Whether this tissue differentiates into bone appears to depend on local physical and chemical factors. Whether the bone formation that may occur in calcified arteries is to be considered an essential stage in the arteriosclerotic process depends on the latitude with which arteriosclerosis is defined. The bone formation is in no way characteristic of arteriosclerosis.

O. T. SCHULTZ.

## Experimental Pathology and Pathologic Physiology

LOCAL TISSUE REACTIONS DUE TO VARIATIONS IN ION CONCENTRATION. K. AKAMATSU, *Beitr. z. path. Anat. u. z. allg. Path.* **85**:348, 1930.

Attempts to determine tissue changes at the interface between living tissue and solutions of different ion concentration meet with difficulty because of rapid changes in ion concentration due to the quick diffusion of the solution into the tissues. To overcome this difficulty Akamatsu devised the ingenious method of preparing small gelatin blocks, each containing a central air bubble. The blocks were hardened in formaldehyde solution to prevent their solution in the animal body, and were washed free from formaldehyde in running water. The solution the action of which was to be studied was injected into the air bubble contained within the gelatin block, and the latter was placed in the peritoneal cavity of a guinea-pig under aseptic precautions. The present report relates to the results obtained with 5 per cent and 2 per cent hydrochloric acid and with 5 per cent and 2 per cent potassium hydroxide. In preliminary experiments, the prepared blocks were kept in a moist chamber at body temperature, and the reaction tested at intervals with indicators. Blocks prepared with 2 per cent hydrochloric acid lost the acid reaction at the surface in fourteen days; with 5 per cent hydrochloric acid in seventeen days. Blocks prepared with 2 per cent potassium hydroxide retained an alkaline reaction for nineteen days, and those with 5 per cent potassium hydroxide for twenty days. Animals into the peritoneal cavities of which the prepared blocks had been introduced were killed at intervals varying from six hours to twenty-one days after introduction. A generalized peritoneal reaction was not set up. Such changes as occurred were limited to the tissues that were in contact with the surface of the block. The acid solutions caused a more rapid and more marked adhesion of the adjacent omentum or mesentery to the surface of the gelatin block than did the alkaline solutions; the latter frequently caused no adhesion whatever. Acid caused a greater and more diffuse emigration of leukocytes to the interface between tissue and gelatin block than did alkali. Leukocytes wandered into the central cavity of the acid blocks; this phenomenon was not observed in the alkaline blocks. The leukocytes in contact with the surface of an acid block had markedly lobulated and deeply stained nuclei that appeared degenerated. The leukocytes in contact with the alkaline blocks had larger and less deeply stained nuclei, with few lobulations.

O. T. SCHULTZ.

THE INFLUENCE OF COAL AND STONE DUST ON TISSUE CULTURES. A. LAUCHE, *Verhandl. d. deutsch. path. Gesellsch.* **26**:107, 1931.

The method of tissue cultures was utilized for the study of pneumoconiosis. Lauche devised a new apparatus that allows one to sprinkle dust on tissue cultures evenly and in the finest particles. By covering part of the culture with small glass bells, the simultaneous dusting of one culture with two different kinds of dust was accomplished. Comparative studies with various substances showed that common coal dust is more avidly engulfed by phagocytes than pure animal coal, and much better than iron dust. The phagocytosis of stone dust is most sluggish.

When coal and stone dust were sprinkled on tissue cultures at the same time, the former increased strikingly the intensity of phagocytosis of the stone particles. This phenomenon is explained by substances adsorbed by the coal which cause a greater motility of the phagocytes.

C. ALEXANDER HELLWIG.

TISSUE CULTURE OF SO-CALLED ALVEOLAR EPITHELIUM FROM TUBERCULOUS LUNGS. F. HENKE and M. SILBERBERG, *Verhandl. d. deutsch. path. Gesellsch.* **26**:114, 1931.

Silver stain does not permit a definite distinction between epithelial cells (alveolar epithelium) and mesenchymal cells of the septums, because the latter cells also take the silver stain. The authors studied, therefore, the behavior



of these cells in tissue cultures. They infected rabbits with bovine tubercle bacilli intravenously and cultivated the lung tissue from two to four days after infection. From observations on the living tissue it was apparent that the so-called large exudate cells in tuberculosis of the lungs behave exactly like mesenchymal cells. First they develop into different forms of histiocytes and finally into ripe fibrocytes. Giant cells can be seen in twenty-four hour cultures, where they form by confluence of exudate cells. The epithelioid cells of the tubercle are derived from the mesenchymal "alveolar phagocytes." The authors accept this term, which was recommended by Seemann in place of alveolar epithelium, because they conclude from their experiments that the alveoli of the lungs are not lined by a continuous layer of true epithelial cells.

C. ALEXANDER HELLWIG.

THE FORMATION OF BILIRUBIN IN TISSUE CULTURES. E. VON BALOGH, S. SUEMEGI and M. CSABA, *Verhandl. d. deutsch. path. Gesellsch.* **26**:118, 1931.

To 730 tissue cultures of different organs a solution of hemoglobin was added, and from four to five days later, the indirect van den Bergh test was performed on the cultures. The formation of bilirubin was noticed from three to four days after the hemoglobin had been added to the cultures taken from the spleen, brain, spinal cord and pericardium of 18 day old chicken embryos and from the iris, the medullary portion of the adrenal glands and the spleen of guinea-pigs. The van den Bergh reaction was negative in cultures made from the heart muscle, cartilage of the ribs, wall of the veins and skeletal muscle. The authors conclude from their studies that bilirubin forms extrahepatically in several organs, most abundantly in the spleen, and that its formation depends on certain cells (reticulo-endothelial cells) present in these organs. Hematoidin and bilirubin are closely related.

C. ALEXANDER HELLWIG.

THE GROWTH RATE OF TISSUE CULTURES IN DIFFERENT PREPARATIONS OF EMBRYONIC EXTRACT. G. BORGER and R. ZENKER, *Verhandl. d. deutsch. path. Gesellsch.* **26**:124, 1931.

Chick embryo extract, carefully dried in the vacuum and dissolved in water, has the same growth-stimulating effect on tissue cultures as fresh extract. After keeping the dried product for five months, the authors found it as effective as fresh extract, when used in fibroblast cultures.

The growth-stimulating substances of embryonic extract are apparently proteolytic ferments. The same processes, such as heating to 60 C. or shaking, which lower the growth-stimulating properties of embryonic extract, were shown to decrease the amount of proteolytic ferments in the extract.

C. ALEXANDER HELLWIG.

THE HYPERPLASIA OF THE UTERINE MUSCLE DURING PREGNANCY. B. FISCHER-WASELS and W. BUENGELER, *Verhandl. d. deutsch. path. Gesellsch.* **26**:129, 1931.

The anatomist Stieve concluded from histologic examinations of the pregnant human uterus not only that the growth of the uterine muscle during pregnancy is due to a hyperplasia of the preexistent muscle fibers, but that new unstriated muscle cells form from fibroblasts, histiocytes and adventitial cells, and even from lymphocytes of the blood. The authors do not believe that the histologic method will ever be able to solve this problem. They examined the uterus of the mouse after the injection of pituitary hormone from the urine of pregnant women. One hundred hours after the injection of the hormone the uterine muscle showed the same hypertrophy as at the end of a natural pregnancy of twenty-one days. By this method the whole process of muscle growth can therefore be studied in short intervals, and it was demonstrated that the uterine muscle hypertrophies exclusively

from preexistent muscle fibers, by very active mitotic division of unstriated muscle cells. Also by using vital stains, Fischer-Wasels and Buengeler found that no other mesenchymal or blood cells participate in the growth of the pregnant uterus.

C. ALEXANDER HELLWIG.

EXPERIMENTAL ARTHRITIS DEFORMANS. F. KLUGE, *Verhandl. d. deutsch. path. Gesellsch.* **26**:216, 1931.

In the rabbit, true arthritis deformans will develop from anaphylactic arthritis. The disease process was studied in many x-ray pictures during the course of the experiment, over a period of three years, and on histologic sections obtained in different stages of the disease.

In rabbits or other animals, when repeated injections of heterologous blood serum, for instance, from the horse or dog, were made into one knee joint, anaphylactic arthritis, which resembled polyarthritic rheumatism, developed. After about six months, without further injection of foreign serum, a chronic hyperplastic synovitis, peri arthritis and osteochondritis developed in the knee joint into which the injection was made. Still later, about one year after the beginning of the experiment, no inflammatory changes were noticeable, but there were destructive processes that resembled, anatomically and in the x-ray picture, typical arthritis deformans: degeneration and even complete loss of the superficial cartilage, vascularization of the cartilage, marginal exostoses, osteoporosis and osteosclerosis.

C. ALEXANDER HELLWIG.

### Microbiology and Parasitology

SPONTANEOUS AND EXPERIMENTAL INFECTION OF PIGEONS WITH *B. AERTRYCKE*. J. R. CASH and C. A. DOAN, *Am. J. Path.* **7**:373, 1931.

The apparently spontaneous development of a fatal disease in undernourished pigeons is reported which is characterized by anemia, marked myeloid hyperplasia of the bone marrow, striking increase of the myeloid elements of the blood and extensive infiltration of the liver and kidneys with myeloid tissue. In addition to these myeloid changes, large, nodular, often necrotic masses of mononuclear phagocytic cells are frequently found scattered throughout the liver, spleen, kidneys and bone marrow. A small, gram-negative bacillus, regularly recovered in pure culture from the blood, liver, kidney, spleen and bone marrow in these cases, has been identified as *B. aertrycke*. In sections, the bacteria are found to be present in the foci of mononuclear cells, but do not occur within the collection of myelocytes. Disease has been produced experimentally in normal pigeons by the intraperitoneal injection of liver emulsion made from naturally infected birds, by intraperitoneal injection of *B. aertrycke* derived from the same source and also by oral administration of single large doses of broth cultures of this organism. Bacteria-free filtrates of broth cultures of *B. aertrycke* have had no demonstrable effect on normal pigeons when injected or administered orally in single large doses. Attention is called to the frequency with which pathologic changes occur in the tissues of apparently normal birds.

AUTHORS' SUMMARY.

CEREBRAL CHANGES IN A FATAL CASE OF CHICKENPOX. H. M. ZIMMERMAN and HERMAN YANNET, *Arch. Neurol. & Psychiat.* **26**:322, 1931.

The patient, aged 13 months, died in convulsions on the fourth day of chickenpox. There were diffuse changes in the ganglion cells (vacuolation of cell bodies) of the cortex, basal ganglions, pons and medulla, lipid accumulation in the adventitial spaces of the blood vessels in the white matter of the parietal and occipital lobes, with areas of demyelination around the blood vessels, and presence of fat granule bodies in the subarachnoid space. Inflammatory phenomena such as plasma cells or lymphocytes were lacking.

GEORGE B. HASSIN.

CATAPHORESIS EXPERIMENTS WITH PROTEIN-FREE SUSPENSIONS OF A BACTERIOPHAGE AND FOWL-POX VIRUS. I. J. KLIGLER, L. OLITZKI and M. ASCHNER, *Brit. J. Exper. Path.* **12**:178, 1931.

Cataphoresis experiments with purified suspensions of *E. coli* bacteriophage indicate that this agent is negatively charged in neutral and slightly alkaline reactions but amphoteric in acid or strong alkaline solutions. In broth suspensions this agent migrated to the positive pole at all reactions. The fowl-pox virus is positively charged on the acid side, is amphoteric in neutral solutions, and carries a negative charge in alkaline solutions. The bacteriophage and fowl-pox virus in purified suspensions are highly sensitive to acid reactions. C. E. CLIFTON.

THE FILTERABILITY OF VACCINIA VIRUS. A. B. GREEN and G. H. EAGLES, *Brit. J. Exper. Path.* **12**:202, 1931.

The authors claim that vaccinia virus passes readily through Berkefeld V filters and to some extent through Berkefeld N and English Berkefeld filters. The  $p_H$  of the virus suspension (6.4-8.4) does not influence the results, and lining the filters with acidified egg white or the use of hormone broth as a diluent is not necessary. These filtrates can be kept in the cold for a week or more without marked deterioration in activity. C. E. CLIFTON.

ULTRAFILTRATION STUDIES ON THE VIRUS OF POLIOMYELITIS. C. E. CLIFTON, E. W. SCHULTZ and L. P. GEBHARDT, *J. Bact.* **22**:7, 1931.

A simple procedure is presented for the preparation of acetecollodion membranes for ultrafiltration studies, and the principles of ultrafiltration are discussed. A method is described for the removal of the lipoids and part of the associated proteins from suspensions of the virus of poliomyelitis. Ultrafiltration studies on this purified suspension indicate that the magnitude of this virus lies below 50 millimicrons in diameter. AUTHORS' SUMMARY.

ETIOLOGY OF TRACHOMA. U. LUMBROSO, *Arch. Inst. Pasteur de Tunis* **20**:137, 1931.

A complete presentation of studies of twenty-eight cases of trachoma is given. The methods of Noguchi, adapted to the cultivation of *Bact. granulosis*, were used, as well as various other procedures designed to recover unknown or previously described organisms. Detailed protocols show the frequent isolation of streptococci and staphylococci, and in no instance the isolation of *Bact. granulosis*. However, in eighteen or twenty-eight cases small bacilli, culturally somewhat different from Noguchi's organism, were recovered. The ordinary pathogenicity of these organisms was nil, and data were not yet at hand concerning their action on the conjunctiva. These organisms were grouped into types A, B and C, based on morphologic and biochemical studies. M. S. MARSHALL.

BACT. GRANULOSIS AND TRACHOMA. CHARLES NICOLLE and UGO LUMBROSO, *Arch. Inst. Pasteur de Tunis* **20**:239, 1931.

*Bact. granulosis* (Noguchi and Olitski strains) have shown themselves, in our hands, to have lost all pathogenicity, both intravenously injected and in the conjunctiva. By the latter route the Olitski organism induced a very light and very transitory follicular reaction; the Noguchi organism produced nothing. In no case was trachoma produced. To these results of rigorous experiment only one objection may be presented, the date of isolation of the cultures used, the aging of cultures altering the virulence of all organisms. AUTHORS' CONCLUSIONS.

SYMPTOMLESS OCCURRENCE OF TUBERCLE BACILLI IN TONSILS WITH RECURRENT ARTHRITIS AND RETROBULBAR NEURITIS. K. AMERSBACK, A. LÖWENSTEIN and E. LÖWENSTEIN, München. med. Wchnschr. **78**:1078, 1931.

Tubercle bacilli were cultured from the tonsils of five patients in a group of fourteen with polyarticular rheumatism. Cultures of the blood were negative. Clinically these patients had no symptoms of tuberculosis. Similar studies in five patients with retrobulbar neuritis, of whom one had the classic symptoms of multiple sclerosis, demonstrated tubercle bacilli in the blood of the patient with multiple sclerosis, and in a second patient tubercle bacilli both in the blood and in the tonsils. The organism in the latter patient was of the avian variety. These results are published preliminary to a more extensive study.

AUTHORS' CONCLUSIONS.

TUBERCLE BACILLEMIA IN DISEASES OF THE CENTRAL NERVOUS SYSTEM. E. LÖWENSTEIN, München. med. Wchnschr. **78**:1080, 1931.

Tubercle bacilli were not cultivated from the blood of 3,000 healthy persons. They were cultured from the blood of patients with chorea, retrobulbar neuritis, multiple sclerosis and schizophrenia without demonstrable visceral tuberculosis.

AUTHOR'S SUMMARY.

RETICULO-ENDOTHELIAL VITAL STORAGE IN EXPERIMENTAL TUBERCULOSIS. W. PAGEL and J. E. GARCIA-FRIAS, Virchows Arch. f. path. Anat. **275**:479, 1930.

The authors report the results following the injection of india ink into guinea-pigs recently infected and superinfected with tuberculosis, the experiments being undertaken primarily for the purpose of studying the responses of the allergic animal. In experimental tuberculosis of the liver, the giant and endothelioid cells of the tubercles stored large quantities of the carbon particles. Storage by these cells was more marked than by the Kupffer cells of the normal and regenerated portions of the liver. The tuberculous process experimentally induced was not altered in any way by repeated injections of india ink over long periods of time or by extirpation of the spleen. Storage was not influenced by the local or generalized allergic reaction to tuberculin. Carbon storage had no influence on the local tuberculin reaction of allergic animals. In such animals the injection of tuberculin caused a more active exudative inflammatory response than did the injection of india ink alone or the injection of the ink and tuberculin in normal animals. The presence of stored carbon appeared to inhibit somewhat the formation of tuberculous granulation tissue following superinfection or a local tuberculin reaction. When india ink and tubercle bacilli were injected at the same time, removal of the stored carbon was more rapid than when the same procedure was carried out in the already infected animal. In the former, abscess formation occurs and the carbon is removed from the area of injection in a relatively short time, whereas in the allergic animal the particles are held for a long time by the cells at the point of injection. Experimental tuberculosis afforded very definite protection against serum anaphylactic shock. The degree of protection depended somewhat on the degree of generalization of the tuberculous process, on the extent to which the lungs and spleen were involved and on the amount of necrosis and caseation. The protection was greater after the generalization of the infection and moderate tubercle formation following subcutaneous injection than after the more rapid generalization and more widespread tubercle formation following intravenous injection of tubercle bacilli. The protection against serum anaphylactic shock could be passively transferred by the injection of organ pulp of tuberculous animals. The serum of such animals had a less marked protective action, and tuberculin, emulsion of tubercle bacilli and brei of normal animals had little or no protective action. The protection against active serum anaphylaxis is ascribed to an altered reaction to toxic substances brought about by the generalization of the

infection and the formation of tubercles. Passive protection is ascribed to protein split products. Exposure of tuberculous animals to roentgen rays, the generalized tuberculin reaction or the injection of iodine compounds, lipoid extracts or drugs on the sympathetic system caused no decrease in the protection afforded by tuberculous infection to serum anaphylaxis. The local tuberculin reaction of the allergic animal sensitized to serum was increased by the simultaneous injection of tuberculin and serum. Serum sensitization did not render normal animals more susceptible to tuberculin.

O. T. SCHULTZ.

THE PORE SIZE AND SIEVE ACTION OF BACTERIAL FILTERS. H. BECHHOLD, *Ztschr. f. Hyg. u. Infektionskr.* **112**:413, 1931.

Evidence is presented to show that the diameter of the pores in a bacterial filter must be from eight to fifteen times as large as the longest diameter of a micro-organism that will pass through the filter. Protective colloids did not increase the filterability of the agents studied. Nonspore formers will grow through filter pores of double their greatest diameter, while spore formers and branching forms will grow through even smaller pores.

C. E. CLIFTON.

## Immunology

NATURE OF THE VIRICIDAL ANTIBODY IN ANTIPOLIOMYELITIS SERUM. E. W. SCHULTZ, L. P. GEBHARDT and L. T. BULLOCK, *J. Immunol.* **21**:171, 1931.

Though viricidal antibodies were easily demonstrated in the serums from human beings and monkeys convalescent from poliomyelitis, no evidence could be elicited to indicate that these antibodies are related to antibacterial antibodies. The presence of complement-fixing and precipitating antibodies specific for the virus could not be demonstrated even in serums from hyperimmunized convalescent monkeys. On the other hand, evidence was obtained that strongly suggests that the antibodies parallel antitoxins in their behavior. Like neutral toxin-antitoxin, neutral serum-virus mixtures may be rendered infective again by dilution with physiologic solution of sodium chloride. Moreover, while an undiluted serum of sufficient titer may render a given quantity of virus material immediately innocuous, when such a serum is diluted a certain minimum of time is required to accomplish the same result.

AUTHORS' SUMMARY.

PROPERTIES OF A BOUILLON FILTRATE OF THE GONOCOCCUS. L. T. CLARK, N. S. FERRY and A. H. STEELE, *J. Immunol.* **21**:233, 1931.

The gonococcus produces an extracellular toxin, when grown in suitable liquid medium that promotes rapid development. This toxin is contained in the sterile bouillon filtrate from young cultures in sufficient concentration to give positive skin reactions in dilutions of from 1:1,000 to 1:1,500. Injection of the toxin into animals stimulates the formation of an antitoxin that neutralizes the toxin in vitro as well as in vivo. Control autolysates and mechanically produced control intracellular extracts give negative skin reactions in low dilutions. An "anti-virus" meeting the specifications of Besredka was not produced in liquid cultures of the gonococcus allowed to incubate for from six to seven days.

AUTHORS' SUMMARY.

A SPECIFIC ANTIGENIC CARBOHYDRATE OF TYPE I PNEUMOCOCCUS. A. WADSWORTH and R. BROWN, *J. Immunol.* **21**:245, 1931.

From type I pneumococci a specific substance, apparently carbohydrate, was isolated. Its properties and antigenic activity are described as follows: Precipitation occurred with type I antipneumococcus serum in a dilution of 1:6,000,000. With type I antipneumococcus serum from which the antibodies against the specific

carbohydrate of Heidelberger and Avery had been adsorbed, precipitation occurred in a dilution of 1:600,000. Complement was fixed in the presence of type I antipneumococcus rabbits serum. Tests for protein were negative, and those for carbohydrate, positive. Mice immunized with the substance were protected against type I pneumococcus infection. It caused fatal anaphylactic shock in guinea-pigs passively sensitized with type I antipneumococcus rabbit serum; also, in guinea-pigs passively sensitized with type I antipneumococcus rabbit serum from which the antibodies against the specific carbohydrate of Heidelberger and Avery had been absorbed.

AUTHORS' SUMMARY.

PYROGENIC REACTION TO ANTIPNEUMOCOCCUS SERUMS. L. D. FELTON and GLADYS KAUFFMANN, *J. Infect. Dis.* **49**:337, 1931.

Chill-producing lots of antipneumococcus immune serum contain material precipitable at a  $p_H$  value of from 4.8 to 5.2, whereas under these conditions good preparations contain little. Chill-producing lots have a slightly higher phosphorus and perhaps a higher lipid content than good ones; the differences are so slight, however, as to indicate that the residuum of these agents in the final preparations may not be responsible for pyrogenic action. Unsatisfactory preparations contain more ammonia and nonprotein nitrogen than do those that are suitable for intravenous injection.

AUTHORS' SUMMARY.

ARTIFICIAL FEVER AND EXPERIMENTAL POLIOMYELITIS. C. W. JUNGBLUT and N. KOPELOFF, *J. Infect. Dis.* **49**:348, 1931.

Noninfectious fever of considerable intensity applied during the period of incubation and maintained over a short period of time is not capable of altering the course of experimental poliomyelitis in the monkey to any significant extent. The same holds true for infectious fever produced by inoculation with *Trypanosoma equiperdum*, when the two diseases are induced simultaneously. With poliomyelitis infection superimposed on existing trypanosomiasis there may be distinct prolongation of the period of incubation of the poliomyelitis infection, undoubtedly indicating some attenuation of the virus. The latter observation is based on one monkey. In poliomyelitis it would seem that the production of a sufficiently intense fever to bring about destruction of the virus in vivo is not possible within a safe zone of raised temperature.

FROM AUTHORS' SUMMARY.

AGGLUTININ ABSORPTION IN UNDULANT FEVER. E. FRANCIS, *Pub. Health Rep.* **46**:2416, 1931.

The chief interest in these studies centers about the serologic reactions of certain cultures of *Brucella abortus* (Bang). A brucella that manifests the cultural character of requiring carbon dioxide for its isolation is *Br. abortus* (Bang), and yet certain cultures of such an organism are shown by agglutination to give the *melitensis* A serologic reaction of *Br. melitensis* (Bruce). Any contention that agglutinin absorption is a reliable test for the differentiation of *Br. abortus* (Bang) from *Br. melitensis* (Bruce) is not supported by these studies. *Abortus* and *melitensis* cultures may be quickly, but only tentatively, separated serologically by their agglutination in one or the other of the following previously absorbed serums: (1) an *abortus* type serum which has been absorbed by a *melitensis* type culture or (2) a *melitensis* type serum which has been absorbed by an *abortus* type culture. The result of such an incomplete test is not to be taken as final evidence, but only as suggestive.

AUTHOR'S SUMMARY.

THE WEIL-FELIX REACTION IN TSUTSUGAMUSHI DISEASE. J. W. WOLFF, *J. Hyg.* **31**: 352, 1931.

The occurrence of the Weil-Felix reaction in a series of forty-five cases of tsutsugamushi disease (type Schüffner) is recorded. The agglutination reaction

was negative in fifteen cases, the other thirty showing a positive reaction with the "Kingsbury" strain of *B. protex* X. The Weil-Felix reaction is not regarded as adequate for differentiating tsutsugamushi disease from scrub typhus, since reactions of high titers (1:1,000) occur in a considerable proportion of cases of tsutsugamushi disease. In the present state of knowledge of the two diseases it is recommended that they should be separated according to the presence or the absence of a primary ulcer and bubo.

AUTHOR'S SUMMARY.

RABICIDAL ANTIBODIES IN RABBITS IMMUNIZED AGAINST RABIES. G. STUART and K. S. KRIKORIAN, *J. Hyg.* **31**:414, 1931.

In the immune serums of rabbits treated with killed, etherized-fixed virus, rabicidal antibodies make an earlier appearance, are present in greater degree and persist a longer time than in the immune serums of rabbits treated with equal quantities by weight of fresh-fixed virus or of killed, carbolized virus.

AUTHORS' SUMMARY.

FOOT AND MOUTH DISEASE IN VACCINE LYMPH. T. VAN HEELSBERGEN, *Ann. Inst. Pasteur* **46**:558, 1931.

Norwegian vaccine lymph no. 980 produced foot and mouth disease, as well as the usual reaction to the vaccine. The possibility of dual properties in a single virus was ruled out by the successful separation and preparation of pure live virus strains, each producing the symptoms known to be associated with it and providing no cross-immunity.

M. S. MARSHALL.

REINOCULATION WITH BCG IN THE GUINEA-PIG. L. BALOZET, *Ann. Inst. Pasteur* **46**:604, 1931.

Repeated intracardiac or intraperitoneal injections of BCG did not alter the virulence of the first inoculation or the histologic changes induced by it. Deaths were frequent following intracardiac injection, and were apparently due to a toxic substance. Repeated subcutaneous inoculations caused an abscess, which, after the third or fourth injection, developed a little more rapidly, but healed rapidly. Repeated subcutaneous injections after an intracardiac injection did not inhibit the healing of lesions due to the latter; following an intraperitoneal injection, healing seemed to be favored by subsequent subcutaneous inoculations.

FROM THE AUTHOR'S CONCLUSIONS.

ANTIGENIC FAILURE OF SPIROCHAETA PALLIDA FROM TESTICULAR CHANCRES. F. PLAUT and H. KASSOWITZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:193, 1931.

The inhibiting effect of the tissue extract is made responsible for the lack of antigenic qualities both in vivo and in vitro. Extracts of spirochetes from cultures that possess antigenic qualities lose these when extracts of various tissues are added.

I. DAVIDSOHN.

THE QUANTITATIVE DEVELOPMENT OF GROUP-SPECIFIC RECEPTORS IN THE SERUM OF THE NEW-BORN INFANT. OLUF THOMSEN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:199, 1931.

The serum of new-born infants contains the same group receptors that are present in the red blood cells. The titer is not lower than that of the serum of adults. In cases of heterospecific pregnancies, the serum of the fetus may combine with the iso-agglutinins of the mother, which have passed through the placenta. This explanation is offered to replace the hypothesis of Hirsfeld of the selective retention of certain iso-agglutinins by the placenta. The rather weak development

of receptors in the red blood cells of new-born infants is another protective mechanism counteracting the deleterious effect of the passage of corresponding iso-agglutinins from the mother.

I. DAVIDSOHN.

DYSENTERY ANATOXIN. M. P. ISABOLINSKI and B. P. KARPATSCHEWSKAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:213, 1931.

To dysentery toxin, 0.5 per cent formaldehyde was added and the mixture kept six days at 48 C. Rabbits immunized with it developed a high degree of resistance to dysentery toxin. The efficiency of the anatoxin was dependent on the strength of the toxin from which it was prepared. The serums of the rabbits so immunized had only weak protective properties.

I. DAVIDSOHN.

THE INFLUENCE OF THE SIMULTANEOUS INTRODUCTION OF VARIOUS ANTIGENS ON IMMUNITY. M. P. ISABOLINSKI, W. A. JUDENITSCH and B. P. KARPATSCHEWSKAJA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:218, 1931.

Guinea-pigs infected with tubercle bacilli and subsequently treated with diphtheria anatoxin did not become resistant to diphtheria toxin as the control animals did. Injection of tubercle bacilli after resistance for diphtheria toxin had already developed resulted in a lowering of this resistance. The injection of BCG, proteus X<sub>10</sub> bacilli and its toxins, antirabic emulsion and scarlet fever vaccine did not interfere with the development of immunity against diphtheria toxin. Children treated simultaneously with scarlet fever vaccine and diphtheria anatoxin developed resistance to diphtheria toxin as estimated with the Schick test.

I. DAVIDSOHN.

THE RÔLE OF THE SKIN IN THE PRODUCTION OF ANTIBODIES AGAINST SPIROCHAETA PALLIDA. F. PLAUT, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:223, 1931.

Very small doses of nonvirulent, pure cultures of *Spirochaeta pallida*, injected subcutaneously or applied on scarified skin, stimulated the development of specific agglutinins and lysins, but no advantage was observed when these routes were compared with the intravenous. Extracts of skin from the areas into which the antigen had been injected did not contain agglutinins, indicating a lack of participation by the skin in the production of antibodies against spirochetes from cultures. Virulent spirochetes from testicular chancres did not produce agglutinins when injected intracutaneously and did not react in vitro with serums prepared against cultural spirochetes.

I. DAVIDSOHN.

THE ACTION OF BACTERIOPHAGES ON THE AGGLUTINOGEN FOR HUMAN RED CELLS IN B. DYSENTERIAE-SHIGA. M. EISLER and A. F. HOWARD, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:473, 1931.

The antigen mentioned in the title, and the Forssman antigen, which are present in B. dysenteriae-Shiga, disappear as a result of the action of bacteriophages on cultures of the organism. The agglutinability of the bacteria by their specific immune serums, which is also lost under the same conditions, is restored by growing the bacteria in alkaline broth; the agglutino-gen for human red cells, however, does not return.

I. DAVIDSOHN.

THE BIOLOGIC SPECIFICITY OF THE FEMALE GERMINAL PLASMA OF THE CHICKEN. RICHARD BREIER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:477, 1931.

Chicken egg-white is species-specific and does not produce lipid antibodies in the rabbit. Immune serum produced in rabbits with injections of chicken egg-yolk



reacts with egg-yolks of other birds, showing this antigen to be organspecific. The egg-yolk immune serum reacts with the homologous egg-white, but not with the egg-whites of other birds; it contains lipid antibodies and reacts with alcoholic extracts of the homologous antigen and of the egg-yolks of other birds. Also general lipid antibodies are present, giving positive complement fixation with the various alcoholic extracts used for the Wassermann reaction. Heterophilic antibodies of the Forssman type (in the form of the proper titer of antisheep hemolysin) are also present. An immune serum produced with boiled egg-yolk reacts with the homologous antigen, with the raw egg-yolk, with its alcoholic extract, and with the egg-yolks and even with the egg-whites of numerous other animals. When used for immunization, alcoholic extracts of egg-yolk behave like haptens. Immune serum produced with alcoholic extracts of lecithin react with alcoholic extracts of various egg-yolks, but not with their watery suspensions. Immune serum produced with duck egg-yolk displays immunologic reactions like those of chicken egg-yolk serum, but does not contain heterophilic antibodies, owing to the absence of the corresponding antigen in the duck.

I. DAVIDSOHN.

THE ANTIGENS OF THE RED BLOOD CELL STROMA. KEIHO KAMADA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **71**:522, 1931.

Chemical fractions of the red cell stroma of the horse displayed differences in antigenic qualities. Complement-fixing antibodies were produced only by the "secondary ether extract," containing phosphatides and cerebroside-like substances. Hemolysins were mainly produced by the "water extract," containing albumoses, nitrogen residue substances, cerebroside, carbohydrates and soluble proteins. The "primary ether extracts," which contained cholesterol-like substances, were distinctly less antigenic. The extraction modifies the antigenic substances, which is also demonstrated by means of proper absorptions. For use in the complement-fixation test, unchanged red blood cells are the most efficient antigen.

I. DAVIDSOHN.

THE ANTIBODIES IN COLD-BLOODED ANIMALS. M. K. EBERT, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:13, 1931.

Frogs produced specific precipitins for rabbit, sheep and rat serums and specific hemolysins and agglutinins for rabbit, sheep and human erythrocytes. The production of nonspecific precipitins could not be demonstrated.

I. DAVIDSOHN.

THE PRODUCTION OF SPECIFIC SERUM WITH PARATYPHOID BACILLI. M. KURODA, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:45, 1931.

Aoki and his associates have demonstrated a specific and a nonspecific variant in paratyphoid bacilli which could, in many cases, be isolated as separate cultures. Specific immune serums were produced with the help of proper absorptions. Their specificity went so far that the serum did not agglutinate strains of the homologous species which did not contain the specific variant. The disadvantage of such too far going specificity was overcome by the production of specific serums that acted equally specifically on the specific and the nonspecific variants of the homologous species, but not at all on heterologous bacteria. To bring out the nonspecific agglutinins, the number of immunizations had to be increased.

I. DAVIDSOHN.

THE CONTENT OF HISTAMINE-LIKE SUBSTANCES IN THE GUINEA-PIG IN PROTEIN SENSITIZATION. K. HOSOYA and K. WATANABE, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:57, 1931.

Immediately following the injection of serum, and during the anaphylactic shock, a decrease of aforementioned alcohol-soluble substances was found in the lung, small intestine and urinary bladder; in the large intestine it was noticeable

only during the anaphylactic shock. A rise of the substance was noted some time after the sensitizing injection, reaching a maximum and then declining slowly while the sensitiveness to the protein persisted without any apparent relation to the level of the histamine-like substance. The liver and striated muscles showed no changes. The temporary nature of the rise points against the hypothesis that the anaphylactic reaction in the guinea-pig is due to the liberation of a histamine-like toxic substance in the tissues.

I. DAVIDSOHN.

THE BINDING OF HEMOLYTIC ANTIBODIES. HANS V. EULER and EDVARD BRUNJUS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:65, 1931.

A study has been made of the influence of the hydrogen ion and of the sodium chloride concentration on the absorption of antsheep hemolysin by the stroma of sheep's red blood cells. The optimum  $pH$  value was found to be about 5.3, both in the presence and in the absence of sodium chloride. At a  $pH$  value of from 6.5 to 6.6, only 10 per cent of the hemolysin was bound if sodium chloride was absent, while if it was present, about 85 per cent was fixed. The influence of the salt was not so marked when lipid extracts of red blood cells were used instead of stroma, but the optimum  $pH$  value remained the same. When the antibody was bound by the lipids, various attempts to separate the two led to a recovery of only small quantities of the former, the presence of serum being a favorable factor for the procedure.

I. DAVIDSOHN.

THE BIOLOGY OF SPIROCHAETA PALLIDA. S. BERGEL, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:93, 1931.

*Spirochaeta pallida* was injected intraperitoneally into rabbits and mice, and the changes in morphology were noted. Similar studies were conducted *in vitro* by mixing the spirochetes with peritoneal exudates rich in lymphocytes and with extracts of lymph glands from an infected animal. Dark field observation and various staining methods were employed. Sections from healing human and from experimental rabbit lesions were also studied. The most important change consisted in the appearance of granules in the course of the spirochetes. Tinctorial degenerative changes were seen with the use of the Giemsa stain. In sections stained according to Levaditi, the spirochetes were less numerous within and in the neighborhood of the lymphocytic accumulations and showed distinct degeneration down to granular disintegration. In agreement with others, the author assumes that *Spirochaeta pallida* shows an outer lipoidal coat, while the center is a lipid-protein mixture. The Wassermann reaction is based on the presence of two kinds of reactive substances: those against specific spirochetal lipoids, and those against the nonspecific lipoids found in tissues infested with spirochetes.

I. DAVIDSOHN.

THE EFFECT OF DIMINISHED CONCENTRATIONS OF SODIUM CHLORIDE ON COMPLEMENT FIXATION. ERWIN NETER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:136, 1931.

The concentration of sodium chloride was diminished to 0.6 per cent in the antigen-antibody-complement mixture. In the sheep's red cells and antsheep hemolysin mixture, a correspondingly higher (1.8 per cent) concentration of sodium chloride solution was used to obtain the concentration of physiologic solution of sodium chloride. The effect was a distinct and uniform intensification of the fixation, when immune serum against heterophilic organ extracts, against lecithin, against serum proteins or against *B. coli* was used with the corresponding antigen. It was shown that the effect was not due to an anticomplementary action of lower concentrations of the sodium chloride solution. The reaction was not less marked at room temperature than in the incubator; it even seemed to be more intense.

I. DAVIDSOHN.

COMPLEMENT IN NORMAL GUINEA-PIG SERUM IN VARIOUS PERIODS OF LIFE. E. FRIEDBERGER and I. GURWITZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:164, 1931.

New-born guinea-pigs had approximately the same amount of complement in their blood as their mothers, while it was absent in fetuses delivered by cesarean section. The complement obtained from guinea-pigs of various ages and weights showed no essential differences in the titers. The old custom of using guinea-pigs of a certain age and weight for complement does not appear justified by the results of this study.

I. DAVIDSOHN.

CAN CONDITIONAL REFLEXES OF PAWLOW STIMULATE THE PRODUCTION OF IMMUNE ANTIBODIES? E. FRIEDBERGER and I. GURWITZ, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:173, 1931.

According to Metallnikow (*Ann. Inst. Pasteur.* **46**:131, 1931; abstr., *ARCH. PATH.* **12**:496, 1931), conditional reflexes are able to stimulate the production of specific antibodies in animals previously immunized, as shown by a rise in the titer. The present study revealed no such effect in rabbits treated with sheep's red blood cells and with cholera vibrios. The slight stimulation shown in the titer of the antibodies was not convincing and possibly was due to individual variations.

I. DAVIDSOHN.

## Tumors

THE EFFECT OF TESTICLE EXTRACT AND OF NORMAL SERUM ON A TRANSPLANTABLE EPITHELIAL TUMOR OF THE RABBIT. F. DURAN-REYNALS, *J. Exper. Med.* **54**:493, 1931.

Extract of rat, rabbit or bull testicle prevents or retards the growth of a rabbit tumor when a mixture of the extract and a tumor cell suspension is inoculated intradermally. Similar mixtures, made with normal rabbit serum instead of testicle extract, give rise to tumors which grow with unusual rapidity. The results are the opposite of those obtained with pathogenic bacteria or filtrable viruses which are enhanced by testicle extract and generally inhibited by normal serum.

AUTHOR'S SUMMARY.

TUMOR SUSCEPTIBILITY AND HEREDITY. C. J. LYNCH, *J. Exper. Med.* **54**:747, 1931.

A male mouse from a strain with a high incidence of spontaneous lung tumors was crossed with several females derived from a low tumor strain. The first generation of offspring was then backcrossed to animals of the original strains. The resulting two groups of offspring differed significantly in the incidence of spontaneous tumors of the lungs. These facts are discussed in relation to others previously discovered. It seems clear from the evidence presented that there are among mice constitutional types which differ in incidence of tumors of the lungs, and that the differences are inherited. The number of genetic factors involved has not been determined. No influence of sex was apparent. The possibility of there being genetic factors that affect tumor age will be dealt with later.

AUTHOR'S SUMMARY.

CARCINOMA OF UTERINE HORN AND INTERSTITIAL PORTION OF FALLOPIAN TUBE. WARREN C. HUNTER and WILLIAM B. HOLDEN, *Surg., Gynec. & Obst.* **39**:746, 1931.

The study concerns a papillary adenocarcinoma in the uterus of a woman, 30 years of age, with menorrhagia almost continuously since the onset of menses, relieved temporarily by curettage, but recurring with increasing severity. The

symptoms simulated those of incomplete abortion, but microscopic examination of the material evacuated from the uterus revealed adenocarcinoma. The uterus on removal was found to contain a papillary carcinoma situated so far laterally in one cornu that it escaped the curet and was found only by making many cuts at close intervals. Microscopic serial sections were necessary to determine whether the growth arose from tubal or endometrial epithelium. The latter proved to be the origin of the neoplasm in what was in all probability at one time a benign polyp. Pressure by surrounding muscle molded the growth, preventing centrifugal extension, except into the uterine cavity. The myometrium was not invaded.

FROM AUTHORS' SUMMARY.

CHORIOCARCINOMA OF THE INTESTINE WITHOUT A PRIMARY GONITAL FOCUS.

A. LAPOINTE, A. CAIN and L. DARFUEIL, *Ann. d'anat. path.* **8**:425, 1931.

The interesting feature in the case is that a thorough examination of the reproductive organs failed to reveal any tumor, whereas the intestines showed multiple foci of the choriocarcinoma. The patient, a woman of 30, died of a septic peritonitis from intestinal perforation about four months following a miscarriage.

B. M. FRIED.

LYMPHANGIOMA OF THE TENDON. R. HUGUENIN and C. OBERLING, *Bull. Assoc. franç. p. l'étude du cancer* **20**:144, 1931.

Two cases of this disease, which occurred in a boy of 3 and in a man of 47, respectively, are well reported.

B. M. FRIED.

A CASE OF PRIMARY SARCOMA OF THE CERVIX. OHLSEN, *Arch. f. Gynak.* **145**:817, 1931.

A woman, 32 years old, had leukorrhea for five months and persistent metrorrhagia and abdominal pain for six weeks. A cauliflower-like growth 4 by 4.5 by 3 cm. arose from the posterior lip of the cervix, was reddish yellow and bled easily on touching. Total hysterectomy was done. No metastases or local invasion were found. The microscopic structure was that of a large round cell and spindle cell sarcoma. Mitoses were absent. The author, as well as Robert Meyer, believed it to be a malignant myoma. The patient was well one year later.

LAWRENCE PARSONS.

THE FUCHS REACTION IN THE SERODIAGNOSIS OF CANCER. B. H. E. CADNESS and C. G. L. WOLF, *Biochem. Ztschr.* **238**:287, 1931.

Using the Fuchs test, the authors obtained in eighteen cases of cancer tested either a positive reaction or an immunity reaction. In sixteen nonmalignant cases the test was negative in 69 per cent; in four cases of stomach ulcer and in one case of nephritis it was positive. Previous irradiation of the patient with x-rays or radium makes the test negative for apparently several weeks afterward. The proteolytic action of cancer serum is diminished after a period of five days in cold storage. The same treatment has no effect on normal serum. Fibrin kept in the icebox for six months is unchanged. The addition of heparin to the serum or the previous washing of the fibrin in 90 per cent alcohol have no effect on the reaction. Previous boiling of the fibrin decreases the proteolysis considerably. The boiled fibrin cannot be reactivated by the addition of enterokinase and calcium chloride. Phosphate buffers of  $pH$  7.4 and 7.1 and borate buffers of  $pH$  7.1 have a lysing action on fibrin, more so at 37 C. than at room temperature. The thereby produced ninhydrin positive substances do not pass through a cellophane membrane. The amount of nitrogen present corresponds with the intensity of the ninhydrin test made with the filtrate. Previous boiling of the fibrin inhibits the action of the

buffers. Precipitation of the serum with aluminum hydroxide leaves the proteolytic ferment in the supernatant fluid. The Fuchs' ferment is therefore probably of a tryptic nature or identical with kathepsin. The ferment is precipitated with the proteins, if alcohol is used.

WILHELM C. HUEPER.

EFFECT OF SODIUM FLUORIDE ON TUMOR METABOLISM. C. SELLEI and J. JÁNY, *Biochem. Ztschr.* **239**:94, 1931.

Tumor fermentation is inhibited by sodium fluoride in dextrose solution, but not in lactate solution. Oxidation is also interfered with by sodium fluoride, but to a lesser degree than fermentation. The fermentation of cancer tissue is decreased, but the respiration is unchanged, at high concentrations of sodium fluoride. In dilute solutions of sodium fluoride, representing from one one-hundredth to one ten-thousandth-normal, the respiration is increased, while the glycolysis is decreased. The tumor metabolism has then an aerobic character. Also, the two heat quotients of cancer tissue are similar to those present in normal tissue.

WILLIAM C. HUEPER.

ACUTE LEUKEMIA WITH A FIVE MONTHS REMISSION. HENRY JACKSON, JR., FREDERIC PARKER, JR., G. P. ROBB and H. CURTIS, *Folia haemat.* **44**:30, 1931.

The case reported concerns a man of 35 years whose blood and bone marrow showed a picture characteristic of acute leukemia. Although at the time of the first admission the patient was critically ill, within a short period he completely recovered. His blood picture too returned to normal and remained so for four and a half months. Such cases, according to the authors, would appear to indicate a non-neoplastic origin of the acute leukemia and would also seem to offer some hope for the ultimate curability of this disease. In acute leukemia there would seem to be a failure of the normal inhibitory factors controlling the growth of the white blood cells, or else a failure of those factors favoring cell maturation. In either event, disappearance of these perversions seems possible, and it is this potential reversibility that would make one so hopeful of the possibility of a cure, even though at present the factors that bring about favorable or unfavorable results are unknown.

B. M. FRIED.

FLUORESCENCE OF CANCER TISSUE. J. KOERBLER, *Strahlentherapie* **41**:510, 1931.

Exposing out surfaces of tumors of the breast to the light of an arc lamp with Goerz-Beck carbons, cancer tissue appeared on the photographic plate in sharp contrast to the surrounding normal tissue due to the fluorescence of the cancer tissue. But a similar effect was obtained in pictures of the wall of a hemorrhagic cyst of the breast and of a tuberculous granuloma of this organ. It is thought that the fluorescence is due to the presence of hematoporphyrin resulting from decomposition of hemoglobin by bacterial action or by metabolic (lactic acid) action of the tumor tissue.

WILHELM C. HUEPER.

METABOLISM OF TUMORS. S. EDLBACHER and W. KUTSCHER, *Ztschr. f. physiol. Chem.* **199**:200, 1931.

All rapidly growing tissues such as tumors, granulomas and embryonic tissue show a characteristic increase in the decomposition of arginine by arginase. Arginine phosphoric acid does not occur in mouse carcinomas. The production of inorganic phosphoric acid from carcinomatous pulp by treatment with bicarbonate is not inhibited by the addition of fluoride, while this takes place, when muscle tissue of normal and carcinomatous mice is treated in the same way. The production of phosphoric acid in tumor pulp is therefore not dependent on the glycolytic processes, but is probably due to the decomposition of phosphatids and nuclear

substances. Nucleic acid added to tumor pulp resulted in a marked production of inorganic phosphorus through the activity of a tumor phosphatase. Malignant tumors of mice, rats and man show this phenomenon in a striking fashion, while the dephosphorylizing process is slower and less extensive in benign tumors, granulomas, etc. The general metabolic effect of the tumor was demonstrated by the fact that animals with cancer show a marked dephosphorylizing reaction in the muscle tissue, which sometimes even surpasses that present in the tumor itself, while the reaction is negative in the muscle of normal animals. Tumor tissue is therefore not only characterized by an activation of the hydrolytic decomposition of carbohydrates, but also of that of arginine and nucleic acid.

WILHELM C. HUEPER.

CARCINOGENIC SUBSTANCES OF ROUS SARCOMA. E. FRAENKEL, *Ztschr. f. physiol. Chem.* **200**:126, 1931.

When the tumor agent of Rous sarcoma is adsorbed to trypan blue and isamine blue, no inhibition of "takes" is observed. The same results were obtained from elutions of "Hansagelb" and different types of thioindigo to which the tumor agent has been adsorbed. Inoculations were, however, negative, when the agent was adsorbed to "Lackorange." It is concluded that the Rous agent must be a chemical substance and not a virus.

WILHELM C. HUEPER.

SKIN CANCER IN RATS AFTER PROLONGED ULTRAVIOLET IRRADIATION. W. PUTSCHAR and F. HOLTZ, *Ztschr. f. Krebsforsch.* **33**:219, 1930.

By means of prolonged ultraviolet irradiation, the writers were able to produce skin cancers in the rat with some regularity. The shortest interval was twenty-seven weeks, but after thirty-seven weeks cancers were observed in all of six animals subjected to the irradiation. There were no apparent relations between the color of the animal and susceptibility to the light. The addition to the diet of irradiated cholesterol was without apparent effect. As concerns tissue changes, those of the connective tissues were purely degenerative, were a feature of the dermatitis and bore no evident relation to the onset of cancer. A specific effect of the irradiation was the periodic regeneration of the hair, with overgrowth of the woolly hairs and formation of giant hairs.

H. E. EGGERS.

THE ADRENALS IN RELATION TO TUMOR GROWTH. H. AULER and W. RUBENOW, *Ztschr. f. Krebsforsch.* **33**:292, 1930.

The effects were studied of the injection of various preparations of adrenal on the progress of implanted tumors in rats. In one series an extract of adrenal cortex was used; in a second series, this was combined with phenols or phenolic acids; and in a third, the animals were given injections of the products of the action of the extract on hydrochinone. Throughout there was observed a high percentage of tumor regressions, and similar results were seen after the injection of the ferment plus red cells, and with the use of a similar ferment derived from *Aspergillus oryzae*. The effect is ascribed to the action of the adrenal ferment on polysaccharide metabolism; with one of their extracts the writers observed a tremendous activation of animal diastase and counteraction of the antiferment effect on this of tumor extracts. The activating material they believe to be of phosphatid character, and its counteractive effect they ascribe to combination with bodies of aldehyde nature.

H. E. EGGERS.

TOBACCO CANCER IN THE RABBIT. A. H. ROFFO, *Ztschr. f. Krebsforsch.* **33**:321, 1931.

By the daily application for eight months of a watery solution of the combustion products of tobacco, Roffo has succeeded in producing a skin cancer of

the rabbit's ear in one of ten animals. Solutions of nicotine and of the distillate from the residue after nicotine extraction were without effect. Roffo lists the possible active substances as essential oils, pyridine and empyreumatic substances, along with a content of potassium carbonate, bicarbonate and nitrate.

H. E. EGGERS.

ON THE CAUSES OF DEATH IN CANCER. L. M. SCHABAD and R. W. GORIAINOWA, *Ztschr. f. Krebsforsch.* **33**:348, 1931.

This study of the changes that may be held directly responsible for death was made on a series of 932 autopsies in cases of cancer. Among these were 209 cases of cancer of the esophagus, 334 of the stomach, 82 of the intestines, 11 of which were primary in the small intestine, 52 of the liver and its adnexa, 87 of the lung and 168 of the uterus. Cancer of the breast was not included. With the cancers studied, metastases were found most regularly with cancers of the lung and liver, and least often with those of the biliary passages and the ampulla of Vater. Bone metastasis was observed in 2.6 per cent of all cases, most frequently with pulmonary carcinoma and that of the lesser curvature of the stomach. In 12 cases, splenic metastasis was observed, 5 of the growths being primary cancer of the lung. Two cancers, both of the colon, occurred in persons under 20; 36—4 of the esophagus, 9 of the stomach, 9 of the colon and 14 of the uterus—caused the death of persons under the age of 30; 13 per cent of all cases occurred in the decade between 31 and 40, with 17 per cent of all cases occurring in persons under this last age. The direct causes of death were in large part dependent on regional involvement, aside from terminal pneumonia, which was present in 20.7 per cent of all cases. Pulmonary gangrene was found frequently in connection with esophageal cancer, ascending infection of the urinary system with uterine and at times with colonic cancer, and suppurative peritonitis, which was the direct cause of death in 17 per cent of cases, in association with abdominal involvement either by the primary cancer or its metastases. Terminal hemorrhage was responsible in only 5 per cent; generalized carcinomatosis, in 20 per cent of lung cancers and in only from 2 to 4 per cent of uterine and esophageal cancers. Exhaustion was a very frequent cause of death in cancers of the esophagus and stomach, but was rare in other cancers.

H. E. EGGERS.

BENIGN TUMORS OF THE SWEAT GLANDS. M. GLASUNOW, *Ztschr. f. Krebsforsch.* **33**:431, 1931.

There are here reported three cases of benign tumors of the sweat glands. The diagnosis is made on the criteria of traces of a double-layered epithelial wall, the presence of a definite basement membrane and of such accessory features as connection with more or less typical sweat ducts. Glasunow states that similar tumors are frequently reported in dermatologic literature under the designation of "Naevus epithelioma-cylindromatosus."

H. E. EGGERS.

ON PRIMARY CARCINOMA OF THE LIVER. M. SOLOWEJ, *Ztschr. f. Krebsforsch.* **33**:442, 1931.

In a primary carcinoma of the liver reported here in a woman, aged 53, Solowej has found three fairly distinct stages of atypical growth: parts with a distinct preservation of organoid structure, those with marked disorder of cellular arrangement and those with pronounced abnormality of individual cells. The case showed tumor metastasis to the periaortal and periportal lymph glands and to the pleura. The author calls attention to a frequently present and somewhat important diagnostic feature: the absence of associated splenic enlargement, which, with the exclusion of such diseases as hepatic syphilis or echinococcus, suggests primary cancer in the liver.

H. E. EGGERS.

CANCER MORTALITY IN THE UKRAINE. A. M. MERKOW, *Ztschr. f. Krebsforsch.* 34:21, 1931.

Despite the fact that the death rate from cancer in the Ukraine has shown a steady increase during the limited period covered by this report, the present rate (1928) is still very low as compared with that of more western countries—69.4 per cent per hundred thousand. The explanation would appear to lie in the relatively preponderant early age distribution of the population. It would appear that in respect to death incidence in general, the Ukraine at present is in the same position as was western Europe a number of years ago, as there is still an unduly high death rate from infectious diseases, particularly tuberculosis and pneumonia. The statistics as to age distribution in deaths from cancer in the Ukraine show no essential differences from those obtained elsewhere; the maximum incidence occurs, as usual, in the sixth decade. As concerns deaths from cancer in persons under 50, these were more frequent in women; of these relatively early deaths from cancer in that sex, 50.8 per cent were caused from cancers of the genitalia and 42.6 per cent from cancers of the breast. In general, the usual age at which death from cancer occurred was higher in rural districts and in the smaller towns—a fact that the writer ascribes to the extensive migration of younger persons from those localities to the larger cities. This difference was not apparent with cancer of the genital organs and of the breast; indeed, with the former relationship was reversed.

H. E. EGGERS.

PRIMARY SARCOMA OF THE SPLEEN. K. SCIESINSKI, *Trav. d. Inst. d'anat. path. d. Univ. de Cologne* 2:436, 1931.

A large round cell sarcoma of the spleen in a man 38 years of age is described. The origin of the tumor, which appears to represent a special group of sarcoma, is traced to the reticulum cells of the reticulo-endothelial system.

### Medicolegal Pathology

SELF-MUTILATION OF DELINQUENTS. NUERNBERG, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 17:431, 1931.

For preventing imprisonment or for gaining certain advantages in penal institutions, delinquents not uncommonly inflict various injuries on themselves. Many kinds of artificially produced morbid conditions have been described and are briefly reviewed. Nuernberg cites fifteen new cases dealing with artificial phlegmon, subcutaneous emphysema of the face, swallowing of the broken handle of a spoon, insertion of needles beneath the skin, simulation of hemoptysis by suction of blood from the socket of a recently extracted tooth, production of acute otitis media by self-injection of aromatic oils, etc.

E. L. MILOSLAVICH.

COMPLICATIONS OF SYPHILITIC AORTITIS. R. POHL, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* 17:443, 1931.

In a period of two and one-half years, one hundred cases of syphilitic mesaortitis were observed, with great predominance of cases in males (87 per cent). In 21 per cent of the cases, the aortic leaflets were involved, and in 36 per cent hypertrophy of the heart was present, the weight of the heart reaching in half of the latter instances 500 Gm. or more. In 27 per cent, aortic aneurysm developed, and in half of the cases perforation resulted nine times within the pericardiac sac. Changes of the coronary arteries, especially stenosis of the main ostium, were observed in 13 per cent of the cases, and cardiac aneurysms were found five times. There is a certain predilection for occlusion of the proximal end of the right coronary artery. One case, involving a 28 year old woman with congenital stenosis of the isthmus of the aorta and diffuse syphilitic aortitis of the ascending portion, is described minutely.

E. L. MILOSLAVICH.



FATAL POISONING WITH THUJA OCCIDENTALIS. G. JUNGMICHEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:449, 1931.

In the literature of the last eighty years, only four cases of poisoning with *Thuja occidentalis* are recorded, the drug having been taken to produce abortion. An additional lethal case is reported, with the chemical and microscopic details. The autopsy disclosed a severe gastro-enteritis and cloudy swelling of all the parenchymatous organs, with beginning fatty degeneration. Extract of *Thuja occidentalis* causes contractions, cramps and congestion of the internal organs, but is not a specific abortifacient. *Thuja occidentalis* is more dangerous than *sabina*. In animal experiments with *Thuja occidentalis*, the liver shows a fatty degeneration of the central and peripheral zones of the acini.

E. L. MILOSLAVICH.

DIRECT FRACTURE OF THE BASE OF THE SKULL. B. PUCHOWSKI, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:487, 1931.

A male dwarf, 50 years old, was attacked and gored by a bull, the horn penetrating the left front side of the neck in an upward backward direction and entering into the skull laterally to the foramen occipitale magnum. Several ribs on the right side were found to have been fractured as a result of the impact of the animal.

E. L. MILOSLAVICH.

A PECULIAR INJURY OF THE SKIN DUE TO AN AUTOMOBILE ACCIDENT. P. HEILMANN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **17**:490, 1931.

A honeycomb-like imprint was found in the skin at the left side of the forehead, due to impact of the radiator of an automobile. [A not uncommon occurrence in this country.]

E. L. MILOSLAVICH.

FATAL PARALDEHYDE POISONING. JULIUS BALÁZS, Samml. v. Vergiftungsfällen **2**:151, 1931.

In the case of a woman, 43 years old, who was found in coma, the diagnosis of paraldehyde poisoning was made mainly on account of the odor of paraldehyde on the breath and in the urine and then on the subsequent demonstration of the presence of paraldehyde in traces of the cerebrospinal fluid and in large quantities in the urine. The amount of paraldehyde taken could not be determined. After death, the mucous membrane of the fundus of the stomach was edematous and ecchymotic (from the open stomach came an intensive odor of paraldehyde); there was a moderate edema of the brain; the lungs were congested, and there were punctiform subpleural hemorrhages. These were the principal changes ascribed to the action of the paraldehyde. Only a few cases of fatal paraldehyde poisoning have been reported, most of them from England, and many of these cases are doubtful.

PHANODORN POISONING. K. WAGNER, Samml. v. Vergiftungsfällen **2**:159, 1931.

Phanodorn is the proprietary name for cyclohexenyl ethyl barbituric acid. The drug is used as a hypnotic. It is also called cyclobarbital. In the case of a man, 52 years old, who was found dead after having taken presumably a very large quantity of phanodorn some ten to eleven hours earlier, the postmortem examination failed to reveal any natural causes to explain the sudden death. Chemical examination revealed a small amount of phanodorn in the stomach, while 21 mg. of phanodorn was isolated from 100 cc. of urine. It appears that the organism rapidly destroys the substance, and its presence in the urine in this case is interpreted to mean that an unusually large amount had been taken.

VOLATILE POISONS. H. ZANGGER, Schweiz. med. Wchnschr. **61**:741, 1931.

In this and other recent articles in the same journal, Zangger describes new experiences and observations concerning volatile poisons, some of which are used as solvents for technical purposes in industry.

### Technical

A DEVICE FOR CLEANING THE BOWEL POSTMORTEM. S. GRÖFF, Centralbl. f. allg. Path. u. path. Anat. **52**:97, 1931.

The author's device consists of a hollow horizontal bar, open on top so as to form a small trough. To the ends of this bar are attached forklike uprights to receive the lugs of a flat weight plate, convex on its lower edge to fit into the groove of the horizontal base bar. In operating the device, the bowel, removed from its mesentery and slit lengthwise, is drawn, mucosa uppermost, between the two bars so that the intestinal content is scraped off. A 300 Gm. weight plate 19½ inches (49.5 cm.) long is used for adult bowels and one weighing 150 Gm. for children.

G. RUKSTINAT.

THE GELATIN TEST FOR LIVER FUNCTION. RUDOLPH MANCKE, München. med. Wchnschr. **78**:1430, 1931.

This test is considered to measure the protein metabolism in patients with disturbances of the liver. It consists in the administration of a gelatin solution by mouth and the determination of amino-nitrogen in the urine at designated intervals. Under normal conditions, the maximum elimination is within the first four hours. In pathologic conditions, the total excretion may be increased or the spacial elimination changed.

AUTHOR'S SUMMARY.

AN IMPROVED METHOD OF OBTAINING RESIDUE STRUCTURES IN MICROSCOPIC SECTIONS BY DRY HEAT. O. SCHULTZ-BRAUNS, Verhandl. d. deutsch. path. Gesellsch. **26**:153, 1931.

Fresh frozen sections were heated in nitrogen in the drying oven at 530 C. After cooling, the sections were enclosed in balsam and examined by dark-field illumination. The structure of the tissue is well preserved in these preparations, and the distribution of the residues in the sections presents instructive pictures. The calcium content of normal and diseased heart, kidney, thyroid gland, brain and benign and malignant tumors is described. In amyloidosis, the deposits of amyloid contained always a considerable increase of residues, which consisted especially of calcium.

C. A. HELLWIG.

AN EXACT METHOD FOR THE DETERMINATION OF COAGULATION TIME. G. ELIAS, Ztschr. f. d. ges. exper. Med. **77**:693, 1931.

An exact method is presented which allows one to follow the progress of the clotting of blood and to represent it graphically. The method is based on the change in the Tyndall phenomenon (tyndallometric effect) which the transformation of fibrinogen into fibrin brings about. The beginning and end of the coagulation, so far as they represent the formation of fibrin, are definitely established in this manner. The method yields a reproducible curve. Such a method of investigation ought to lead to a deeper insight into the process of coagulation in general. The exactness of this method invites further studies concerning the change of fibrinogen into fibrin. With the newest type of photometer it was possible to carry out the foregoing experiments with 0.3 cc. of blood. For investigation of the human plasma this is a great advantage.

SANDER COHEN:

# Society Transactions

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## CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, Oct. 12, 1931*

R. H. JAFFÉ, *President, in the Chair*

SKODA AND ROKITANSKY. R. H. JAFFÉ.

About a hundred years ago, morbid anatomy had very modest quarters in the General Hospital of Vienna. Its home was in a small building in one of the wide, tree-covered courts of the hospital. The room that served as pathologic-anatomic theater was just large enough to hold the wooden autopsy table and a few chairs. Most of the time artificial light was necessary, supplied by an oil lamp fastened to the ceiling by a string. In this room Johann Wagner, associate professor of pathology and prosector, gave his demonstrations. The room was packed with students and young physicians, who eagerly followed Wagner's autopsies and interesting demonstrations, since good lectures were scarce in the days of declining fame of the first great Viennese medical school.

Among the students who never missed a lecture in pathology were two shy young men who soon became friends. They had come to the big city from small Bohemian towns, and each was bashful because of the slight Slavic accent of his German. The younger one, Josef Skoda, the son of a blacksmith at Pilsen, had traveled to Vienna on foot. The older one, Carl Rokitansky, was better situated. Though his father, a government employee at Königgrätz, had died when Carl was but 8 years of age and had left the family in poor financial circumstances, an uncle offered some support, and Carl made the trip to Vienna in a peasant's wagon.

Skoda and Rokitansky had selected the study of medicine because of inclination. Full of enthusiasm, they soon recognized that morbid anatomy formed the basis for the understanding of the nature of diseases. It was the common interest in pathology which bound them together, and the acquaintance made in the small morgue soon grew into a friendship which was destined to give much to the science of medicine.

Though young in years, Skoda and Rokitansky were critical enough to recognize the deficiencies of the medical teaching of their days. In a letter to his relatives, Rokitansky called the lectures a "lot of words without real background." The whole conception of clinical medicine centered about symptoms, and little significance was attributed to the observation of the patient, the objective findings and the underlying organic lesions.

In their endeavor to find a better basis for their studies, Skoda and Rokitansky became acquainted with the achievements of the French school. Jean Nicolas Corvisart des Marets and Pierre Adolphe Piorry had revived and improved the method of percussion which had been described first by the Viennese physician, Leopold Auenbrugger, but which soon had been forgotten. The genial Theophile Hyacinthe Laennec had discovered auscultation and had correlated the acoustic findings with exact observations at the autopsy table. Compared with the dogmatic and somewhat mystical medicine that prevailed at this time, the new methods of the French meant a tremendous progress. These new ways became the starting point of Skoda and Rokitansky, who, however, did not simply apply and confirm what the French had found, but went much further, and their clear and critical minds deepened and supplemented what the esprit of the French had created.

After graduation, the pathways of Skoda and Rokitansky parted. Rokitansky stayed with Wagner at the morgue, first as an unpaid resident, later as a paid assistant. When Wagner died in 1832, Rokitansky substituted for two years,

and in 1834 was officially appointed as Wagner's successor. Ten years later, at the age of 40, Rokitsansky was made professor of pathologic anatomy and head of the newly created department of pathology. Pathology was now a compulsory subject of the medical curriculum.

While Rokitsansky never felt the inclination of making practical use of his knowledge at the patient's bedside, Skoda's field was in the hospital wards. In 1833 Skoda became resident physician in internal medicine at the General Hospital, in 1840 attending physician, and in 1846 head of the department of internal medicine. In his capacity as a clinician, Skoda remained a regular visitor of the morgue. By watching Rokitsansky's autopsies and by correlating the anatomic findings with his clinical observations, Skoda learned his internal medicine. He had nothing else to teach him, except the book of the French authors. After the autopsies, he discussed his observations and conclusions with Rokitsansky, and in these discussions Rokitsansky usually was the donor. Virchow once called Rokitsansky the first true descriptive pathologic anatomist, and compared him with Linné. This characterization, however, does not do full justice to Rokitsansky, who questionably was more than a merely descriptive investigator, though he was the founder of the modes of expression in descriptive pathology. In his first publication dealing with the internal intestinal incarceration, Rokitsansky described not only the anatomic changes, but also tried to explain the symptoms, and suggested therapeutic procedures. Many of his later publications revealed a similar trend. In later years, Rokitsansky made himself familiar with the new science of histopathology, and he recognized that studies of morbid anatomy had to be completed by experimental observations on the living animal and by chemical research. When in 1875 Rokitsansky resigned from the chair of pathology, he stated in his farewell address that his aim had been to do his work as pathologist in the spirit of a science the chief purpose of which was to stimulate clinical medicine. "As pathology has given to the knowledge of clinical medicine a broader and firmer foundation, it has itself penetrated into the pathologic histology. In order to complete itself through research on the living animal it has created experimental pathology."

After Skoda had empirically learned auscultation and percussion, he became much interested in the physical background of the variations of the sounds, and reconstructed artificial models in order to determine the conditions under which the different qualities of sound occurred. His talents in mathematics and physics helped in experiments which taught him that the classifications of qualities of sound given by the French were much too complicated and lacked a physical explanation. About the same time, a British physician, Latham, had reached similar conclusions.

The outcome of Skoda's studies was a monograph entitled "Treatise on Percussion and Auscultation," published in 1839. It took a long time before this booklet, printed on blotting paper, found the recognition that it deserved, for it undoubtedly belongs to the classics of the German medical literature. It is interesting to note that in this book the pulsation of the cervical veins and the accentuation of the second pulmonic sound are mentioned for the first time.

Though different in their fields, Skoda and Rokitsansky reveal great similarity in their work. In both there is the tendency to simplify, to bring order into their respective sciences, to group similar conditions together and to separate what does not belong together. Both had an intuitive feeling for causal relations. Of the two, Rokitsansky was the stronger personality. He guided Skoda in the right direction. Rokitsansky's high opinion of Skoda is shown in his letter of recommendation written to the medical faculty on the occasion of filling the vacant chair of internal medicine: ". . . he (Skoda) is predestined as a teacher because of the brilliance of his mind, the correctness of his judgment, a man unbiased by the authority of others and by speculation, a shining light to the student, an example for the ambitious and a stronghold for the hesitating."

Skoda's and Rokitsansky's new ways in medicine met with much opposition among the older members of the Viennese medical profession, who had been

educated in the spirit of the all-explaining symptomatology. The opposition merely strengthened the bonds of friendship. Skoda was Rokitansky's most ardent supporter, one of the first to recognize his greatness. But, in spite of the strong influence which Rokitansky exerted on Skoda, the latter did not give up his own judgment, and he did not follow Rokitansky where he was wrong. Thus, Skoda did not accept Rokitansky's theories of the dyscrasias in which he tried to trace the diseases to abnormal compositions of the blood, theories which were so severely criticized by the young Virchow. It was perhaps due to Skoda's influence that Rokitansky gave up his conception of the dyscrasias and came to the recognition that the organic lesions are the causes of the diseases.

In later years, Rokitansky, the philosopher, distinguished sharply between idealisms as a philosophy of life in the sense of Kant and Schopenhauer, and the strict materialism that should be the philosophy of research.

Rokitansky's greatest contribution to medical science was his textbook on pathology in three volumes, the first edition of which appeared in the years from 1840 to 1846. In the introduction to this first edition, Rokitansky stressed the importance of the materialism in science: "A right conception of power and matter teaches that there is no power without a substratum. Every phenomenon is the expression or the result of the matter which passes from one stage into another one." Rokitansky's textbook was based entirely on his own experiences, and he was unbiased by the preexistent great monographs on pathology by Morgagni, Baillie, Meckel and Andral.

Many other publications came from Rokitansky's tireless pen, such as those on: the spontaneous rupture of the aorta, the diverticulum-like dilatations of the trachea, the strictures of the intestinal tract, the penetrating gastric ulcer, the deformities of the spine and pelvis, the combination and exclusion of pathologic processes, the anatomy of goiter, the important diseases of the arteries, the papillary carcinoma, the growth of connective tissue and its relation to inflammation, the proliferation of connective tissue in the nervous system, and the defects of the septums of the heart.

Rokitansky's great interest in philosophic questions is shown in his two academic lectures: "The Independent Value of Knowledge" and "The Solidarity of Animal Life." In 1863 Rokitansky was called to the State Department of Education as adviser in questions of medical education. In this capacity he exerted a brilliant influence on the development of the medical faculties of Austria. He created special chairs for experimental pathology and medical chemistry, founded the first psychopathic clinic in Austria, called Billroth to Vienna and Klebs and Breisky to Prague. As a member of the Austrian senate, he urged the separation of the school from the church.

Skoda was a physician with all his heart. He divided his time between the clinic and his consulting practice. He wrote little, but what he wrote revealed his deep interest in pathologic anatomy. His greatness expressed itself in his lectures and bedside clinics. Ever and ever again he impressed on his audience the importance of morbid anatomy as the basis of clinical medicine, and he considered his teaching as part of Rokitansky's work. His clear and simple diction enabled him to transmit to his pupils his trends of thought, and every demonstration of a case not only afforded a wealth of information, but was a great stimulus to independent thinking. Instead of writing papers and books, Skoda became the founder of a great school of clinicians famous for their keenness of observation and correctness of diagnosis, which has often been called the pathologic anatomic school of medicine. I do not know of a better example of the great progress that can be accomplished by the close cooperation between clinician and pathologist than is revealed by the lives of Skoda and Rokitansky.

#### DUAL CARCINOMAS, PRIMARY IN THE LUNG AND IN THE PROSTATE. P. A. DELANEY.

A white man, aged 64, had a primary carcinoma of the lung. The postmortem examination demonstrated an extensive carcinoma of the left lung, the cells of

which were small and formed no regular structural units. The metastases were widespread and extensive. A second carcinoma was found in the prostate; the tumor infiltrations also were anaplastic cells.

COMBINED CONGENITAL EXSTROPHY OF THE FEMALE URINARY BLADDER AND CLOACA. GRAHAM A. KERNWEIN.

The report will be published in the ARCHIVES.

TULAREMIC LEPTOMENINGITIS. ARTHUR R. BRYANT and EDWIN F. HIRSCH.

The report was published in the ARCHIVES (12:917, 1931).

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*Regular Monthly Meeting, Nov. 9, 1931*

R. H. JAFFÉ, *President, in the Chair*

THE ANTIGENIC RELATIONSHIP OF THE LIPOIDS FROM BRAIN AND TESTICLE.  
JULIAN H. LEWIS.

Rabbits immunized with emulsions of beef testicle produce antisera that react with the alcoholic extract of testicle and brain. They do not react with the alcoholic extracts of the other organs. Positive reactions are obtained with the brain and testicle of every species tested. Antibrain and antitesticle sera react with cholesterol, while antiliver and antikidney sera do not react with this substance. None of the antisera reacted with lecithin.

HEMOCHROMATOSIS AND PRIMARY CARCINOMA OF THE LIVER. S. R. ROSENTHAL.

The article was published in the ARCHIVES (13:88, 1932).

BILATERAL DIFFUSE CORTICAL NECROSIS OF THE KIDNEYS. A. WOLFSON.

Rare instances of bilateral diffuse cortical necrosis of the kidneys, occurring in the absence of thrombosis of the larger renal vessels, have been reported from time to time. Most of the thirty-five reported cases occurred with pregnancy, but approximately ten occurred with scarlet fever, diphtheria, dysentery, etc. The first case of which I have found any record was reported by Friedländer in 1883 and occurred in a 36 year old woman convalescent from scarlet fever.

A colored woman, 35 years of age, a sextipara, was admitted to a medical ward of the Cook County Hospital on April 28, 1931. She died within a few minutes after reaching the ward. The diagnosis in the examining room was pneumonia. Three months before admission, the patient, then about three months pregnant, had weakness which became progressively worse. Two weeks before admission, she began to have severe headaches and emesis. Two days later, or three days before admission, she had vaginal bleeding. A physician terminated the pregnancy. The symptoms became markedly worse. She had almost complete anuria, which persisted until death. During this time only small quantities of turbid, bloody urine were passed. Two days after the onset of the anuria, she had spots before the eyes and blurred vision, which lasted until death. There were also chills and fever, a foul-smelling vaginal discharge and dyspnea. The patient was conscious until death, which occurred rather suddenly.

The postmortem examination was performed by Dr. Jaffé. The most striking findings were in the kidneys. Other changes were hypertrophy of the heart (weight, 550 Gm.), postpuerperal uterus with pseudomembranous endometritis (Staphylococcus in cultures) and focal necrosis of the liver. The indican content of blood obtained at autopsy was approximately 5 mg. per hundred cubic centi-

eters. There was a peculiar localized cortical necrosis of the kidneys in intimate relation with certain segmentary vascular changes affecting particularly the interlobular and arcuate arteries. The interlobar and larger renal vessels were almost entirely free. The glomerular tufts and the afferent vessels as well as the interlobular arteries were widely dilated and filled with erythrocytic debris, and most of the interlobular vessels with thrombi. The arcuate arteries were patent, but many had marked intimal proliferative changes and advanced organizing thrombi. The medullary tissue and the marginal zones of cortical tissue were well preserved. In relation to these zones, toward the necrotic regions, was extensive calcification of the tubules and glomeruli. Not all blood vessels had the same changes. Some of the interlobular arteries distally were distended and contained fibrin and fairly intact erythrocytes. In relation to these vessels were islands of tissue with distinct tubular and glomerular details. In other regions the entire interlobular artery and even the associated arcuate artery were extensively thrombosed. Where arcuate vessels were thrombosed they had the most extensive organization. The latter type of interlobular artery was in relation to those areas of cortex that had the most severe necrosis.

Bacterial stains failed to reveal the presence of organisms in the tissues.

#### THROMBOSIS OF THE HEPATIC ARTERY WITH SUDDEN RESISTANCE TO INSULIN IN A DIABETIC PATIENT. HERBERT POLLACK AND ESMOND R. LONG.

I. K., a white man, 56 years of age, was diabetic for nineteen years and under dietary control. In 1924 he had a midhigh amputation for a gangrenous left foot. He first came to the University of Chicago Clinics in January, 1931, with a gangrenous toe on his right foot. Supracondylar amputation was done, depriving him of his remaining leg.

He was observed frequently in the out-patient department and once in the hospital for two days. On June 15, 1931, he was admitted with the complaints of vomiting, some abdominal pain and persistent sugar in his urine. Physical examination revealed only marked acidosis. The blood sugar was 410 mg. per hundred cubic centimeters. The urine contained much sugar and acetone. The first six days he received from 90 to 130 units of insulin and was brought out of acidosis. On the seventh day a mild acidosis suddenly developed, and 470 units of insulin was given. The acidosis was improved, but the blood sugar remained between 200 and 400 mg. per hundred cubic centimeters. The next day he received 540 units of insulin, and the blood sugar became 184 mg. per hundred cubic centimeters. Because of an enlarged thyroid gland and the resistance to insulin, compound solution of iodine was given by rectum the following day. Four hours later the blood sugar fell to the levels found in a state of shock. Three hundred grams of dextrose was given intravenously, subpectorally, orally and by proctoclysis before the blood sugar rose to 54 mg. per hundred cubic centimeters. It continued to rise in spite of the administration of 260 units more of insulin.

The next morning the patient was sitting up in bed and was rational. A definite odor of acetone was noticeable on his breath. The blood sugar was too low to be estimated at this time. In spite of forced administration of dextrose, he died in a few hours.

The necropsy findings, as reported here, were unexpected. While mesenteric thrombosis was diagnosed, the gangrenous pylorus with perforation and peritonitis were not suspected. The extensive thrombosis of the vessels arising from the celiac artery was another surprise.

The most significant lesions disclosed by the necropsy were: advanced generalized atherosclerosis; arteriosclerotic atrophy of the pancreas; recent thrombotic occlusion of all branches of the celiac artery; necrosis of the wall of the lesser curvature of the stomach; acute generalized peritonitis; thrombotic occlusion of the pancreatic branches of the hepatic artery and recent anemic necrosis of a considerable portion of the pancreas; multiple recent anemic infarcts of the liver; recent and encapsulated anemic infarcts of the spleen; advanced atherosclerosis

of the coronary arteries of the heart, with recent infarction of the myocardium of the left ventricle; arteriosclerotic atrophy of both kidneys; recent thrombosis of the right renal artery; recent anemic infarct of the right kidney; marked atherosclerosis of the arteries of the leg stumps and organizing thrombus of the right common iliac artery and its branches; mural thrombosis of the first part of the thoracic aorta; colloid goiter; hypertrophy of the prostate; hypertrophy of the wall of the urinary bladder; slight acute hypostatic bronchopneumonia on the right.

The condition of the celiac artery and its branches is illustrated in the accompanying diagram. The splenic, left gastric and hepatic arteries were thrombosed, and the rounded end of the thrombus projected into the celiac artery. The splenic artery was a dilated, tortuous cord, thrombosed throughout. The left gastric and gastro-epiploic arteries were thrombosed in their beginning portions, but not in the distal parts, which were presumably patent because of anastomoses with branches from the middle colic and esophageal arteries. The hepatic artery and all its branches (right and left hepatica propria, right gastric, gastroduodenal and an accessory pancreatic artery) were thrombosed throughout their length. The

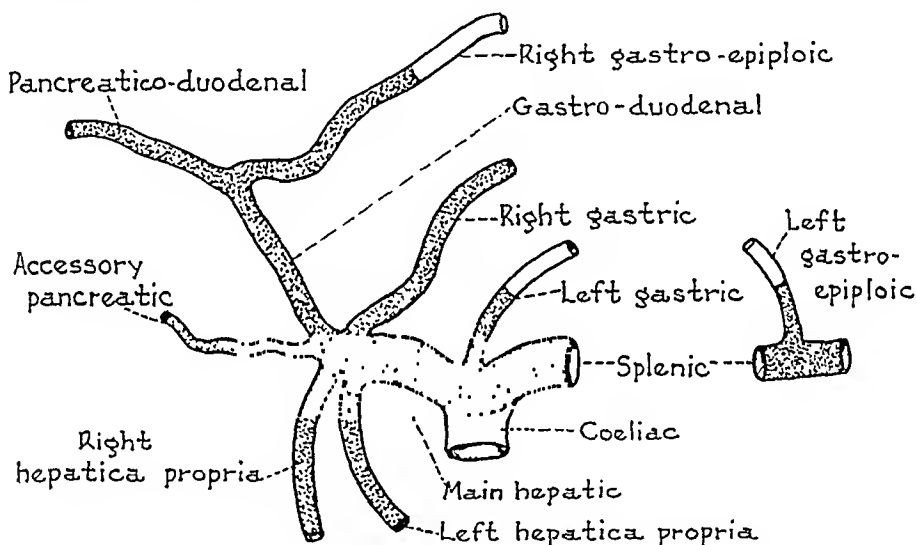


Diagram to show the thrombosis of the celiac artery and its branches.

pancreaticoduodenal artery was completely occluded, but the right gastro-epiploic, like the left gastro-epiploic and left gastric arteries, was thrombosed only in its beginning portion, distally being patent by anastomoses with other vessels. The right gastric (pyloric) artery was thrombosed throughout its length. It led directly into a gangrenous region, 7 by 13 cm., in the pylorus and pyloric portion of the lesser curvature of the stomach. Spontaneous perforation of this resulted in fatal peritonitis.

The pancreas weighed only 55 Gm. Large portions were necrotic. The arteries in the substance of the gland were hard. Microscopically, there was extensive arterial thrombosis with necrosis of the gland substance and leukocytic reactions around the necrotic regions. The arteries in general were thickened, and the fibrous tissue was increased throughout the gland. Hyaline changes were present in many of the islets of Langerhans.

The liver weighed 1,800 Gm. In the dome was a discolored, yellow region 6 by 8 cm. at the surface and of slightly softer consistency than the surrounding hepatic tissue. Other smaller areas of similar changes were scattered throughout the liver. Microscopically, early degenerative changes were seen in the hepatic cells of these regions, including loss in nuclear staining and swelling and vacuolization of the cytoplasm. Few leukocytes were present, and the affected areas were



not demarcated. The cellular glycogen of such regions was abundant and apparently not different from that observed normally. The gallbladder was connected by adhesions with the right colic flexure and appeared in no way modified by the thrombosis of the hepatic artery.

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*Regular Monthly Meeting, Dec. 14, 1931*

R. H. JAFFÉ, *President, in the Chair*

THE RÔLE OF NUTRITION AS A FACTOR IN THE PREVENTION AND TREATMENT OF DENTAL DISEASES. M. T. HANKE, C. M. MARBERG and W. H. TUCKER.

Data obtained by numerous investigators indicate that changes in the structure of the teeth may be associated with diets deficient in vitamins. Changes in dental structure are obtained in young animals when they receive diets deficient in either vitamin D or vitamin C. A demonstration of the histologic changes produced in the teeth and in the surrounding structures when guinea-pigs are fed diets deficient in vitamin C was made by means of natural color photographs of the sections. Experiments on guinea-pigs also show that rapid repair of the injured tissues occurs when the scorbutic animals are given orange or lemon juice in addition to the basal diet.

A survey of the literature and a study of 400 private patients conducted in collaboration with the Chicago Dental Research Club indicate that most persons are ingesting diets that are deficient in vitamins and, possibly, in other constituents. Diseases of the oral tissues, such as gingivitis, periodontitis or dental caries, are most frequently found in persons whose diets are markedly deficient in one respect or another. A deficiency in vitamin C appears to be most common. A deficiency in vitamin D is not uncommon. Usually, then, one is confronted with at least a dual deficiency. On the assumption that many of the pathologic dental conditions are in some way associated with nutritional deficiencies, we have, for three years, been advocating a very liberal diet that should contain all of the constituents that are essential for the development of healthy body tissue.

On this dietary regimen, gingivitis that is not associated with calculus disappears fairly rapidly (in from thirty to sixty days) in most cases. Calculus must, of course, be removed in any case if a complete recovery is to be obtained. Loose teeth frequently become tight. Alveolar bone is not regenerated. A large proportion of the pyorrheal alveoli have become free from pus, and most of the loose teeth have become satisfactorily tight. This takes time; in some cases, as long as two years. Teeth that are very loose, owing to a loss of alveolar bone, do not become tight and can hardly be kept free from pus.

About 85 per cent of patients who had formerly been afflicted with rapid progressive decay have remained free from dental caries for from two to three years. Some patients have been refractory, and in some the amount of caries has decreased, but the process has continued. Nutritional deficiency is probably not the only cause of dental caries; but it appears to be an important factor.

We have never observed a case in which a spontaneous recalcification of a carious lesion has occurred. Although we have numerous cases in which the cavities have not become larger, we have no cases in which the cavities have become smaller.

These preliminary findings have been subjected to a careful recheck under controlled conditions on a group of 422 children at Moosehart, Ill. The children ranged in age from 10 to 17 years—323 regular children and 99 controls. The 323 regular children were observed for one year on the standard Moosehart diet, which was somewhat deficient in vitamins A and D and quite deficient in vitamin C.

All but the small approximal cavities were filled at the beginning of this period. Gingival conditions were recorded by means of natural color photography. We

also obtained fourteen film dental roentgenograms on all children (at twelve month intervals), determined the calcium content (total and ultrafiltrable) and the total phosphorus content of the blood serum (at six month intervals), determined the hydrogen ion concentration in various regions in the mouth, studied x-ray pictures of the wrist (at twelve month intervals), made a study of the bacterial flora of the mouth, and determined the weights and heights of the children at six month intervals.

During this control period, 70.5 per cent of the children had some form of gingivitis; in 80.83 per cent carious lesions developed.

After one year on the standard diet, the cavities were again filled. No other dental work was done. The standard Moosehart diet was then augmented by the addition of 16 ounces (473 cc.) of orange juice and the juice of 1 lemon per day (this is the test period).

All of the gingivitis that was not associated with calculus disappeared during the one year test period (citrus fruit juice). In some of the cases with calculus great improvement occurred, and in some the condition healed entirely, in spite of the calculus. In most cases, however, the presence of calculus effectively prevented improvement.

Dental caries was arrested to the extent of 50 per cent and retarded to the extent of 16 per cent. We consider caries to have been arrested if cavities developed during the one year control period and did not develop during the one year test period.

This shows that the carious process can be influenced by some constituent of orange and lemon juice in addition to the standard diet. This constituent may be vitamin C, but our work does not prove this. The diet is not as adequate as that which we have been advocating to private patients; the results were not as good. This fact may be very important.

The boys at Moosehart show a gain in weight and an increase in height that are below the normal standards in some age groups (standard Moosehart diet). The addition of orange juice to the diet led to an increase in weight and height such that the values, for this one year period, were often in excess of the normal standards. Orange juice therefore supplied something that materially enhanced the rate of growth of the boys. This increase cannot be attributed to the slight increase in the number of calories ingested, because the children at Moosehart received an adequate number of calories.

The relation of total weight to height is approximately correct in all groups even on the standard Moosehart diet.

The determinations of the calcium and phosphorus of the blood serum which were conducted at six month intervals on every child have shown that (1) the values are all within the normal range; (2) the values obtained in the spring are slightly lower than those in the fall, which may indicate a deficiency in vitamin D, and (3) the values for total and ultrafiltrable calcium and for total phosphorus are no different in the group in which carious lesions developed than in the group in which these lesions did not develop. The development of carious lesions is not associated with deviations in the calcium and phosphorus content of the blood serum.

We have made a careful study of the occurrence of aciduric micro-organisms (that are also acidogenic) in the mouths because of the prevalent belief that carious lesions are initiated by the decalcifying action of acids, which, in turn, are produced by the metabolic action of bacteria on carbohydrates. Three groups of such micro-organisms were demonstrated, namely: streptococci, which were almost always present; *Lactobacillus acidophilus*, present as discussed later, and yeast, which was present in about 33 per cent of the mouths. *L. acidophilus* appeared consistently in the mouths of some children that were not afflicted with dental caries. This micro-organism is not, therefore, an obligate producer of dental caries. This statement is equally true of streptococci. *L. acidophilus* was found most frequently and consistently (but not invariably) in mouths that contained numerous carious lesions.

The accumulation of food débris in cavities may provide conditions that are favorable for the localization of this micro-organism in the cavities.

Our bacteriologic studies have done nothing either to prove or disprove the theory that dental caries is the result of the action of bacterial products on the dental substance.

Dental caries is most apt to occur in mouths of children who have had considerable caries in the past. Filling the old cavities does not, apparently, prevent the development of new cavities. A definite tendency toward dental caries exists, which appears to influence the progress of the disease during adolescence. We might consider this tendency to be due to heredity. This is a proper consideration if we enlarge the definition of heredity to include a varied ability to remain healthy in the face of adversity. Since all of the children at Moosehart are living under identical conditions, the existing state of deficiency, dietary or otherwise, will affect some children more unfavorably than others only if certain children require more of a given thing, or things, to remain healthy than is required by others.

It has long been claimed that the teeth decay most rapidly during the period of puberty. This is, indeed, the case; but the increase, during the period of puberty, is hardly as great as one would have expected.

An improvement has occurred during the test period in all groups irrespective of any previous tendency toward dental caries or of age. The addition to the diet of this one constituent has in many cases overcome the untoward effects of heredity and of puberty. This would strongly suggest that the citrus fruit juice contains something with which these children have previously been insufficiently supplied. The quantity of citrus fruit juice administered was so large that it proved adequate even for those who showed the greatest evidence of deficiency.

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Dec. 10, 1931*

LEILA CHARLTON KNOX, *President, in the Chair*

SARCOMA-LIKE TUMOR OF THE PLEURA. MENDEL JACOBI and HERMAN BOLKER.

A white boy, 6½ years of age, with a history of rheumatic fever and rheumatic mitral disease, had had for several months cough, intermittent fever and a suggestive bulging of the left upper part of the chest anteriorly. A diagnosis of pleurisy with effusion was made. At autopsy, a large mass was present in the left side of the chest, completely covered by visceral pleura, adherent to parietal pleura, and compressing the left lung against the vertebral column. The center of the mass was necrotic and hemorrhagic. It was composed of irregular groups of round and spindle-shaped cells. Definite invasion of blood vessels was shown. There was no invasion of pulmonary parenchyma. The heart presented a chronic and subacute rheumatic endocarditis, with Aschoff bodies. The pathologic diagnosis was: sarcoma of the pleura, visceral, subserous type, and rheumatic pancarditis.

### DISCUSSION

PAUL KLEMPERER: These tumors are interesting because of the type of cell concerned. In the group of cases which Dr. Rabin and I described a year ago we were dealing mainly with the spindle cell type of sarcoma, except for one case in which there was an embryonal type of connective tissue present. These round cells in general suggest a more malignant type of tumor. This might account for the invasion of tissue in this instance. I might say, however, that one of our cases which at the first examination presented the picture of a typical

fibrosarcoma showed in its latest stages, at an operation performed about four years after the first one, a marked variation of the type of cell, and in this instance there were also metastases found within the other lung four years after the first operation, at which the entire tumor could not be removed. It is remarkable that even with these metastases the patient is still living today, which indicates that tumors arising from the subpleural tissues are not as malignant as other types of tumors, particularly not as malignant as pleural tumors that originate from the mesothelium. The case is unique because of the age of the patient. Generally these tumors occur at a much later stage of life.

ALFRED PLAUT: May I ask how the reticulum of this tumor looks? Have you used any silver stain or Mallory's stain?

HERMAN BOLKER: We have not used a silver stain. There was little fibrous tissue surrounding the tumor. Where the cells were in groups there were no fibers.

ALFRED PLAUT: It might be interesting to try a silver stain.

#### MYCOTIC ANEURYSMS AT THE ROOT OF THE AORTA IN SUBACUTE BACTERIAL ENDOCARDITIS. DAVID PERLA and (by invitation) SAM ROSEN.

An instance of mycotic aneurysms in the sinuses of Valsalva in a case of subacute bacterial endocarditis (*Streptococcus viridans*) was reported. A boy, aged 17, presented the clinical and pathologic findings of an old rheumatic lesion of the aortic and mitral valves with aortic insufficiency, mitral insufficiency and embolic lesions in the kidneys, brain and intestinal tract. The pathogenesis of the mycotic aortic aneurysms in subacute bacterial endocarditis was discussed. Thrombosis by embolization of small branches of the coronary arteries supplying the root of the aorta is followed by anemic necrosis and septic infarction of the wall of the aorta, with subsequent abscess formation. Pouching of these areas occurs with rupture and the production of aneurysms by dissection through the necrotic area.

In the instance reported in this paper, embolic thrombosis of vasa vasorum was followed by a periaortitis and mesaortitis with abscess formation in the adventitia and media and subsequent rupture into the lumen of the aorta. The presence of organized thrombotic occlusion of vasa vasorum with recanalization outside the wall of the old aneurysm further suggests the embolic origin of the lesion.

#### DISCUSSION

IRVING GRAEF: I should like to ask if Dr. Perla feels that antecedent rheumatic aortitis has been ruled out as an underlying basis for the development of some of these aneurysms in subacute bacterial endocarditis.

DAVID PERLA: It may be that healed rheumatic lesions in the aorta, particularly the intimal lesions, predispose to the implantation of vegetations on the intima of the aorta from the lesions on the aortic valve in subacute bacterial endocarditis. In this case, a small, healed intimal rheumatic lesion a few centimeters above the aortic cusp was noted, but no others were observed. I do not believe that rheumatic infection played any direct rôle in the formation of the aneurysms.

#### STUDIES ON THE HISTOLOGIC DISTRIBUTION OF FAT AND LIPOIDS IN VARIOUS DISEASES OF THE KIDNEY. A PRELIMINARY REPORT. IRVING GRAEF and (by invitation) HENRY HORN.

An analysis is presented, based on histochemical examination, of the distribution of fat and lipoids in various lesions of the kidney. The classification adhered to is that of Volhard and Fahr. The final pathologic diagnosis of the renal conditions is based on study of sections stained with hematoxylin and eosin, by van Gieson's and Weigert's elastica methods, with McGregor's modification of

Mallory's connective tissue stain, and by Heidenhain's azan carmine methods. Tissues of patients succumbing to acute infections and of patients dying in congestive heart failure have also been studied. The tissues of the kidneys, lungs and liver have been examined in each of ninety-nine cases, that of the kidneys with special reference to the distribution of fat and that of the lungs and liver for signs of stasis. Edema and ascites have also been looked for. This has been done to correlate signs of stasis with deposition of fat in the kidney.

For the study of fat, staining with sudan III counterstained with hematoxylin, treatment with osmic acid, staining with Nile blue sulphate and use of the polariscope have been the methods employed. Several frozen sections of formaldehyde-fixed tissue from each case were so studied.

In four of the eight normal kidneys studied, scanty intracellular deposits were found in the distal collecting tubules. There were no signs of stasis in either the lungs or the liver. Eight cases of congestive heart failure, six with advanced rheumatic valvular disease, one with severe coronary sclerosis and one with syphilitic aortitis, are included. In each were found marked signs of stasis, and in each, prominent fatty deposition in the epithelium of all varieties of tubules. Four cases in young adults, in whom an acute infection was the cause of death, presented prominent fatty deposits in tubules of all types associated with signs of stasis in sections of the liver and lungs.

One case of acute, diffuse glomerulonephritis with minute glomerular deposits revealed prominent fatty deposits in the tubules. There were marked signs of passive hyperemia in the liver and lungs.

In eleven cases of subacute, diffuse glomerulonephritis the vessels showed slight change. In ten, which were of the extracapillary form, the crescents always contained more striking deposits than the tufts proper. Nine of the eleven revealed prominent tubular fatty change. In seven of these there were associated definite signs of stasis elsewhere. Only two of the group with moderate glomerular deposits and signs of stasis showed slight tubular alteration. In the remaining two cases, tubular changes seemed commensurate with the glomerular alterations.

Of seven cases of chronic, diffuse glomerulonephritis, three in which the kidneys weighed more than 250 Gm. together revealed slight vascular deposits. In the four in which the kidneys weighed less than 250 Gm., the vascular deposits were more marked, in some instances entirely obscuring the lumen. The glomerular deposits were more prominent than in the cases of subacute nephritis. In only three of the group were tubular deposits commensurate with the changes in the other units. These cases also presented definite signs of stasis in the lungs and liver. In two cases with contracted kidneys there were slight tubular deposits even in the presence of marked vascular and glomerular change. In these, the signs of passive hyperemia were equivocal. In the remaining two cases with moderate vascular and glomerular deposits and slight focal tubular changes, the evidence of stasis was scanty.

The nephrosclerotic group consists of forty-three cases comprising two groups. The first group includes kidneys weighing together between 250 and 350 Gm.; the second group, kidneys weighing between 100 and 250 Gm. In the first group, nineteen specimens were examined. Fatty deposits were found in all units—consisting of subendothelial deposits in the afferent arterioles and of basal intimal deposits in the interlobular branches, and in the glomeruli varying from fine to globoid intracellular and extracellular masses of fat. In ten of the nineteen, the tubular deposits were far more prominent than the vascular or glomerular changes, and in all ten there was prominent evidence of stasis. In two, in addition, there were edema and ascites. In the remaining nine cases, only one instance with slight tubular deposit revealed definite signs of hyperemia. The remainder presented tubular changes corresponding to the alteration in the other units.

In the fourteen cases with kidneys weighing less than 250 Gm. together, the fatty deposits were more marked. An apparent association between passive hyperemia and tubular deposition was also observed here.

In four cases of the so-called end-stage of nephrosclerosis were found marked glomerular and vascular deposits and but slight tubular deposits. In three of the

four there were slight signs of stasis. In the fourth case there was slight pigmentation of the central hepatic cords. There was no edema in any instance.

In six cases of malignant nephrosclerosis, the accelerated form of Löhlein and Klemperer, marked fatty deposits were found in all units, contrary to the belief of Herxheimer. In every instance there were striking and widespread signs of stasis in the liver and lungs. In two there were edema and ascites.

In seven cases of focal embolic glomerulonephritis with striking deposits in the epithelium of all varieties of tubules, there were definite signs of passive hyperemia. In four, there were edema and ascites. Five cases of amyloidosis presented slight vascular and glomerular deposits and moderate tubular deposits. In three, there were definite signs of passive hyperemia. In one case with scanty deposits, there were no signs of stasis. The fifth case with marked fatty deposits in all units showed only slight signs of passive hyperemia.

Four cases of mercurial nephrosis showed scant focal globular deposits close to regenerating cords of tubules. The associated signs of stasis were slight. A fifth case with antecedent arteriolar nephrosclerosis and signs of passive hyperemia had marked tubular deposition of fat.

One instance of acute interstitial nephritis occurring in pneumococcal pneumonia presented slight vascular internal glomerular deposits. Tubular epithelial deposits were frequently encountered in the cells of the collecting tubules. There were signs of congestion in the liver and lungs.

We have found sudan III combined with hematoxylin a satisfactory stain for fat and lipoids; Nile blue, sulphate, very irregular and inconsistent, and valueless in older material; examination by means of the polariscope showed doubly refractile substance in the chronic nephropathies, usually scant in amount and invariably in association with sudanophil material.

Fatty substances have been found in a wide variety of conditions. While fat phanerosis can explain histologic increase or appearance in organs showing no chemical increase, as Wells has stressed, passive hyperemia occurs in many patients with moderate or severe nephropathies and must be taken into account in explaining the appearance of fat in uriniferous tubules.

#### DISCUSSION

PAUL KLEMPERER: While I think that one must agree that passive congestion, particularly in heart failure, is very frequently or almost always associated with a marked fatty infiltration of the renal tubules, so much so that we speak of the characteristic appearance of the kidney in heart failure, I should like to ask Dr. Graef whether he believes this is the only cause for deposition of fat. I do not think that one should generalize, because there is a marked fatty change in mushroom or phosphorus poisoning, which hardly can be explained on the basis of passive congestion. I think we have to consider other reasons, particularly metabolic causes, for the deposition of fat in these kidneys.

IRVING GRAEF: I should like to make only two points in addition to what Dr. Horn has said. In the case of benign nephrosclerosis with heart failure it is striking that the tubular deposition of fat is just as marked in the intact tubules as in the atrophic tubules. Many atrophic tubules shed their lining epithelium after going through the stage of deposition of fat. The more severe arteriolar lesions are undoubtedly associated with a degree of tubular injury that must be related to the etiologic factor, which is as yet unknown, but in these cases, too, the degree of heart failure is so striking that the association cannot be disregarded. In the amyloid kidney there is a totally different picture. In our material, three cases were associated with signs of passive hyperemia. Of these, two presented edema and ascites. The mechanism for this edema and ascites, we recognize, may be related to a special dysfunction of the kidney somewhat analogous to true lipid nephrosis. In the fourth case with signs of passive hyperemia, we found practically no fat in the tubules. In the fifth case with no signs of hyperemia, we found abundant fat.

The study is still in progress, and we should be glad to receive any suggestions. We are particularly disappointed in the use of Nile blue sulphate. We found it a most unreliable dye, and we could not depend on the results, particularly since the aging of the material makes considerable difference in the consistency of the results.

ENDEMIC AMEBIC DYSENTERY IN RESIDENTS OF NEW YORK CITY. PAUL KLEMPERER.

Eleven cases of amebic dysentery observed within the last five years were reported. Five of the patients were foreign-born, and probably had contracted the disease in their native country, though two of them had been residents of New York City for the last four and eight years, respectively. The other five patients were natives of the United States and had lived all their lives in or around New York City; one of these patients had been a resident of Missouri. The last patient was born in Austria, but had been living in New York City for the past thirty years. Only in four of the eleven cases was a clinical diagnosis of amebic dysentery confirmed by examination of stools. In the other seven cases, the condition was revealed only at the autopsy table or microscopically in surgical specimens removed from the patient. The clinical diagnosis in these cases was carcinoma of the large intestine in three instances, peritonitis, acute appendicitis and ulcerative colitis each in one. One patient who died from bilateral lobar pneumonia showed amebic dysentery as an incidental finding.

DISCUSSION

LOUIS FAUGERES BISHOP, JR.: In a study of amebiasis, sixty cases occurring at Bellevue Hospital were received. About twenty-five per cent of the patients had no history of ever having been out of New York City, so far as could be determined. For that reason I feel as Dr. Klemperer has said, that amebiasis is probably far more frequent in New York City than any of us who are practicing here realize. It is not always an easy matter to make a diagnosis, and I think Dr. Klemperer has brought out the fact that all of us should be more on the lookout to make the diagnosis of amebiasis before autopsy. I think with a more careful examination of stools, using the warm stage, we shall be able to distinguish these cases earlier, and by so doing, be able to help these patients.

W. GRETHMANN: I think Dr. Klemperer is justified in stressing the necessity of looking for patients with *Entamoeba histolytica*. In the tuberculosis wards of Bellevue Hospital in the course of the last year I had an opportunity to see three cases of entamoeba infestation. All three patients, as far as I can remember, were residents of New York and there was nothing in their histories to reveal that they had lived in the South. The first case was combined with tuberculosis; intestinal symptoms were present. The latter were naturally attributed to tuberculosis, and the presence of *Entamoeba histolytica* was not suspected clinically, but was found post mortem. Soon after this there was a second case in which the pulmonary lesion and the picture as a whole did not seem to indicate so severe an intestinal lesion as the patient showed clinically; the feces were searched for *Entamoebae*, and they were found. The third case was that of a patient suffering apparently from an abscess of the lung. Operation was done, and it was found that the abscess was too large for successful drainage and the wound was closed. At autopsy, the large intestine was considerably involved by ulcerated lesions, but by far the larger lesion was found in the liver. Practically the whole of the dome of the liver was transformed into a crater-like lesion measuring about 15 cm. in diameter. There were direct ulceration and penetration of the diaphragm and empyema on the corresponding side, together with ulceration of the lower lobe of the right lung. I think the three cases serve to substantiate Dr. Klemperer's opinion that infestation with *Entamoeba histolytica* is not uncommon in New York.

## Book Reviews

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**Studien über die Entstehung und den Verlauf der Lungenkrankheiten.**  
By Dr. N. P. Tendeloo, o. ö. Professor der allgemeinen Pathologie und der pathologischen Anatomie, Direktor des pathologischen Instituts der Reichs-universität Leiden. Second edition. Paper. Price, 26 marks. Pp. 219, with 6 illustrations. Munich: J. F. Bergmann, 1931.

Of the first edition entitled "Studien über die Ursachen der Lungenkrankheiten," the "Physiologischer Teil" with a separate bibliography was published in 1900; the "Pathologischer Teil" was published two years later; the completed work was dedicated to van Heukelom. At this time the author was prosector in the municipal hospital at Rotterdam. For a number of years he has been professor of pathology in the university at Leiden and is well known through his textbook "Allgemeine Pathologie," first published in 1919, with a second edition in 1925. These studies of pulmonary disease, revised after about thirty years, are so completely rewritten that only traces of the original text remain. A new format with smaller modern type, larger printed space, some condensation and the total pages cut to less than half of the first edition have all combined to produce an attractive monograph. Except for slight rearrangement and new captions for some chapters and minor subdivisions, the plan of the original work is closely followed.

The physiologic studies are of forces responsible for the capacity of the lungs; modifications resulting from localized dilation or compression; variations in different parts of the lungs of their normal respiratory capacity; energy of air currents, the blood and lymph flow in the lungs, and finally, practical applications of these with especial attention to their relation to the inhalation of dust and to drowning. Discussion of the mechanics of normal respiration, of the ease of movements involved in quiet breathing and the tonus and relaxation of respiratory muscles convey impressions of rhythm balanced as marvelously as is that of the heart, or the recurring symmetrical contractions of medusae. Convincing reasons are advanced for believing that the paravertebral cephalad parts of the lungs are least, and the portions at the outside about half-way between the apex and base are most, concerned with breathing. The account of what takes place when persons drown leaves little to be desired, and the description of the gross appearance of the lungs is especially to be commended. In considering the forces involved in drowning, explanations are made of why groups of air sacs dilate intermittently until they explode, why others lessen in size to even complete atelectasis, what determines distribution of the air and inhaled fluid, and which changes are primary and which secondary. The questions are stated, and the answers are set forth in deliberate, entertaining discussions interspersed with the results of investigation by others, his own experiments and illustrations and other details of the useful apparatus he devised. The studies of drowning and inhalation of dust provide convincing evidence of regional diversities in normal lungs in the movement of air, blood and lymph, as well as in respiratory capacity.

From this foundation of physiologic studies an easy and natural transition is made to studies of pulmonary disease. The author's style of separate, pointed questions prevails in the chapters on bronchopneumonia, lobar pneumonia and pulmonary tuberculosis; for each disease the same six questions serve as texts. They deal with where the inflammations start and how they spread, the causative bacteria, the rôle of secondary infections or other factors, the manner and nature of subsequent extensions of the disease, the way location in the lungs modifies what follows, and routes by which the infectious agents arrive where the disease begins. The term lobar is replaced by diffuse; broncho and lobular, by localized. The peculiarities of localized pneumonias of typhoid fever, bubonic plague, of



nurslings, the exanthematous diseases of childhood and other diseases are described. Importance of the quality of the virus is admitted, but emphasis is given to susceptibility to infection, allergy and other systemic and local conditions. Tendeloo believes that too much attention has been given to the germs. He maintains that diffuse (lobar) pneumonia is a sequel to infection somewhere else, that the inflammation in the lungs is at first deeply seated, that for its development an unusual hyperemia must first occur where the fibrin is subsequently precipitated, and that the demarcation of this hyperemia rather than anatomic barriers determines the limitation of the initial consolidation. These and other views are apparently well corroborated by the principles governing the functions of normal human lungs presented in the first half of the work. But they are also, surprising to relate, confirmed by the features of diffuse pneumonia in horses, cattle, dogs and goats. Interesting comments are made about the anatomic and clinical differences of diffuse pneumonia due to its location. Here as elsewhere the author occasionally pauses to introduce personal observations; at one period he practiced medicine for eight years.

In the masterly account of pulmonary tuberculosis, also much the longest of these studies, are assembled the salient features of contributions to this subject made by the author during almost two decades. They also served in a similar manner for the article "*Pathologische Anatomie*" in the "*Handbuch der Tuberkulose*" by Brauer, Schröder and Blumenfeld, now in its third edition.

Skilfully arranged in discussions of the six questions formulated also for nontuberculous pneumonias, this more recent review is a brief, comprehensive statement of modern problems regarding pulmonary tuberculosis. The author designates as "ideal" two of the four forms of tuberculosis he recognizes, possibly because they are more readily understood. These are both primary infections steadily progressing on the one hand to healing; on the other, to death. A third variety is also a primary infection, which becomes clinically evident, and then more or less latent with a variable subsequent course. The fourth form is ordinary phthisis with its chronicity, recurring periods of quiescence, disseminated and diffuse exudates, its cavities and systemic infection. One must read this chapter carefully to form a proper estimate of this authority's skepticism as to the rôle of allergy in tuberculosis. It is a distrustfulness comporting well with the caution and critical attitude manifest in the emphasis he gives to the inconstancy of virus potency and host susceptibility, the absence of methods of estimating such factors quantitatively and to the numerous exogenous and endogenous influences to which they are subjected. He returns again and again to these mutations and the impossibility of applying set ideas about them to the disease in any single patient. To conclude that any particular tuberculous focus in the lungs is due to a fresh exogenous reinfection, there are many requirements that must be fulfilled. It is fair to assume that the author's necropsies possess elements of finished execution and that the requirements he mentions are investigated as a routine. Restrictions of a book review prevent mention of the edifying discussion in this chapter of many other problems of pulmonary tuberculosis. The reader's interest is continually stimulated by definite suggestions pertaining to their investigation.

In the chapters on pulmonary emphysema and atypical forms of pneumonia no effort is made to answer stipulated questions. In pointing out how different parts of the lungs may be permanently, abnormally dilated by inspiratory and expiratory overdistention, Tendeloo again reverts to principles of structure and function discussed in the first part of the monograph. The differences between senile atrophy and senile emphysema of the lungs and their causes are brought out in strong relief. With other atypical pneumonias the singular and anomalous changes of the lungs in influenza are described, also the interstitial pneumonia studied by MacCallum, the aspiration pneumonia of the newly born, asthenic pneumonia and the pneumonia of old age. That occurring with psittacosis is barely mentioned probably because reports of its remarkable histologic changes appeared about the time this monograph was in press. A good index and a splendid bibliography are at the end. The references to American investigations are perhaps more numerous

than is the average in German publications. Advanced students in medicine with time for leisurely adventures in its literature will find this monograph profitable reading; for teachers, it is full of source material; for investigators of pulmonary diseases, it is a prerequisite.

**Ein bewegtes Gelehrtenleben. Erinnerungen und Erlebnisse, Kämpfe und Gedanken.** By Otto Lubarsch. Pp. 606. Berlin: Julius Springer, 1931.

This autobiography is of special interest to pathologists and medical men in general. The author pursued an active and varied career in pathology during the height of prewar German scientific development as well as during the war and afterward. The story of his experiences and activities under such dramatic circumstances starts in Berlin and ends in Berlin, where he was born in 1860. Though of Jewish origin, his parents were Christian in religious belief. Lubarsch has regarded himself always as a true and loyal German in the best sense, but he has been subjected more than once to bitter and unwarranted attacks on the score of a fancied antisemitism (see chapter 19). He prepared himself for medicine in the university at Leipzig and studied medicine in Jena, Berlin, Heidelberg and Strassburg, taking his doctor's degree in 1883. In Strassburg he came in contact with Jacques Loeb, the physiologist, and Eduard Kaufmann, the pathologic anatomist. After short periods of assistantship in Bern under Kronecker, the physiologist, and in Giesen under Bostroem, he served for a year and a half as assistant to Ponfick in Breslau, where he received a good training in gross pathologic anatomy. While in Breslau a row with another assistant led to a duel with pistols, in consequence of which he became, so to speak, outlawed. Unfavorable reports hindered him in obtaining a permanent appointment. He worked first with Neisser and then in Virchow's institute in Berlin. Here he first met Ostertag, the veterinary pathologist, with whom he started a few years later the publication of the Lubarsch-Ostertag *Ergebnisse der allgemeinen Pathologie und pathologischen Anatomie des Menschen und der Tiere*, which has appeared regularly ever since.

After working for some months in the zoological station at Naples, Lubarsch next became assistant to Klebs in Zurich, where he remained for about two years. Here he worked with Hanau on experimental tar cancer but without positive results. A good description is given of the condition in the laboratory and of the personality of Klebs, who at that time was so preoccupied with his tuberculocidin and its practical use that he resigned in the fall of 1891. From Zurich Lubarsch migrated to Rostock, where he remained for several years as assistant to Thierfelder. Here he had the great luck to be married happily. This period, which otherwise was marked by disturbing experiences and controversies, ends with his removal to Posen, where he was head of the pathologic division in a newly created hygienic institute. A principal part of his duties here was to make postmortem examinations in various hospitals in Posen. He was now mainly a hospital pathologist. The complicated racial, linguistic and religious conditions in Posen in relation to scientific medicine form the subject of an entertaining account. By 1905, the situation in Posen was unbearable, and Lubarsch now spent first one year as pathologist to a hospital in Lichtenfelde, where Bismarck's physician, Schweninger, was the head, and then a year in a like position in Zwickau, whence he was called to the newly created academy for practical medicine in Düsseldorf. Five years later, in 1912, he became professor of pathology in Kiel, and in 1917 he succeeded Orth in Virchow's chair in the University of Berlin, where he remained until retired on reaching the age limit. In 1917, he became editor of *Virchow's Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, which he still conducts. His son was killed in the World War. The conditions during the war and after, particularly as they affected him and his work, are described graphically and without restraint. The principal episodes of the Berlin period are the struggle to maintain the right to make autopsies; the work of the committee of which he was chairman, to study and report on the so-called

Friedmann cure for tuberculosis, and the Kutisker case, in which he was accused of violating the pledge of professional secrecy because he told his class the name of the person whose organs he was demonstrating.

Separate chapters are devoted to medical education, editorial policies, political interests, travels and science in general. The account of his travels in Russia to attend medical meetings is noteworthy. At the International Medical Congress in Budapest in 1909 he again met Jacques Loeb, whom he had not seen since his days in Strassburg. The impressions of this meeting led to the following comments: "Äusserst lehrreich war es und ist es mir geblieben, dass er [Loeb] mir sagte, er würde einen Ruf an die kleinste deutsche Universität annehmen, denn die Abhängigkeit von den Geldgebern der amerikanischen Universitäten wäre drückend und auf die Dauer unerträglich. In einem solchen Rufe standen damals die königlichen und grossherzoglichen Universitäten Deutschlands; ob Loeb auch unter den jetzigen Verhältnissen, wo die Unabhängigkeit der Hochschulen durch die sozialistische Herrschaft in immer zunehmendem Masse bedroht wird, den gleichen Wunsche geäussert hatte, ist mir zweifelhaft. Dass sich aber die Verhältnisse in Amerika seitdem nicht wesentlich geändert haben und die fast ausschliessliche Einstellung der meisten Amerikaner auf den Dollar deutschen und europäischen Gelehrten deutscher Vorbildung auf die Dauer das Leben dort verleidet, habe ich noch vor Kurzem erfahren, als der vor einem Jahre verstorbene hervorragende russische Histologe und Pathologe A. Maximow bei seinem letzten Aufenthalt in Berlin mir die gleichen Klagen vorbrachte wie Loeb, und wieder etwas später ein österreichischer an einem der grössten nordamerikanischen wissenschaftlichen Institute angestellter Pathologe über die ganze geistige Einstellung der amerikanischen Bevölkerung beweglich klagte." (It was and will remain to me extremely instructive that he [Loeb] told me he would accept the offer of a professorship in the smallest German university, because the dependence on the donors of the American universities was oppressive and in the long run unbearable. Such was the reputation of the royal and grand ducal universities of Germany at that time; whether Loeb would have expressed this wish under the present conditions, when the independence of the universities is more and more threatened by the socialistic regime, is doubtful. However, that the conditions in America have not changed much since then and that the almost exclusive focusing of the majority of Americans on the dollar make life in the long run unbearable for the German or European scientist of German education, I have experienced again recently, when the outstanding Russian histologist and pathologist A. Maximow, who died a year ago, at his last sojourn in Berlin, voiced the same complaints to me as Loeb, and again a little later an Austrian pathologist, who holds a position in one of the greatest North American scientific institutes, deplored the entire cultural attitude of the American people.)

Lubarsch writes clearly, seriously and earnestly. He describes persons and conditions frankly; some would say too frankly. Himself he portrays as robust, gruff and industrious, and he does not slur the fact that associates found it difficult to get along with him. Somehow he seems to fail to discuss his own researches adequately. But he does set forth his hopes and fears, his predilections and prejudices, his successes and his thwartings, and the portrait strikes one as real, as true to life.

## MEDIAL DEGENERATION IN THE AORTA OF THE RABBIT PRODUCED BY DIPHTHERIA TOXIN \*

G. LYMAN DUFF, M.D.

George Brown Memorial Fellow, University of Toronto, 1931

TORONTO, CANADA

The remarkably damaging effects of diphtheria toxin on various organs of the body have long been recognized, and the changes due to it have been the subject of a great deal of study both in man and in various experimental animals. However, the degeneration occurring in the media of the aorta and in the large arteries of animals subjected to injections of diphtheria toxin has received but little attention. Degenerations of the same type produced in the arteries of experimental animals by other agents (e. g., epinephrine) have been much more thoroughly investigated, and their study has proved of definite value in the interpretation of certain varieties of lesions in human arteries. The present report, accordingly, has as its object the confirmation and amplification of the rather scanty literature dealing with arterial degeneration in rabbits due to diphtheria toxin.

Mollard and Regaud<sup>1</sup> in 1897 were the first to note the occurrence of lesions in the aortas of animals that had received injections of diphtheria toxin. They made this observation in only two animals. One was a rabbit that had been given four doses of toxin intravenously over a period of five months. The animal died eight days after the last dose. Autopsy revealed a diffuse lesion of the aorta, which was apparent in the gross specimen. The intimal surface was rugous, pale and checked with fissures. The second animal was a guinea-pig which had received a single dose of toxin subcutaneously and had died spontaneously about twenty months later. The aorta of this animal was severely involved from its origin to its bifurcation, the lesion being most pronounced in the upper part of the thoracic aorta and the last part of the abdominal aorta. The renal arteries showed a similar change. Unfortunately no microscopic examination of the vessels was recorded. The authors were judiciously hesitant in drawing conclusions from so few observations, but reported their failure to find lesions of a similar nature in the aortas of a number of healthy animals

\* Submitted for publication, Oct. 3, 1931.

\* From the Department of Pathology and Bacteriology, University of Toronto.

1. Mollard, J., and Regaud, C.: *Compt. rend. Soc. de biol.* 49:756, 1897.

from their own laboratory stock. It would seem from their description of the lesions compared with later observations that at least in the rabbit the changes in the aorta were produced by the injections of diphtheria toxin.

Klotz<sup>2</sup> mentioned the occurrence of lesions in the aortas of rabbits that had been given intravenous injections of diphtheria toxin. He gave no detail of the experimental procedure, but indicated that the lesions produced were of a purely degenerative character and confined to the media, affecting principally the first part of the aorta, where thinning of the arterial wall, calcification and aneurysmal dilatations were apparent in the gross specimen. He also pointed out the similarity between these lesions and those produced by injection of epinephrine, barium chloride or digitalin. Furthermore, he was able to show that these lesions were not dependent entirely on a rise in blood pressure as had been inferred from Josué's original experiments with epinephrine, but rather on a toxic action directly affecting the media of the aorta. The action of diphtheria toxin was confined to the same elements of the wall of the vessel as was that of epinephrine, barium chloride or digitalin. These damaging agents differed only in degree of toxicity.

Bailey<sup>3</sup> published a more detailed report on the effect of diphtheria toxin in producing medial degeneration of the arteries of rabbits. He injected, intravenously, diphtheria toxin alone into fifteen rabbits and toxin and pituitary solution into seventeen. Some animals received a single dose, while others received repeated doses. In four animals receiving diphtheria toxin alone, lesions of the aorta developed in from eight to twenty-eight days. In four receiving diphtheria toxin and pituitary solution similar lesions developed in from seven to twenty days. In both groups, some of the animals showed changes in the large branches of the aorta similar to those found in the aorta itself. In the gross specimen, the vessels showed dilatations, thinning out of the media and frequently cracks extending through the intima into the medial coat. All the vessels were somewhat rigid and stiff to the touch, whether calcification was present or not. The lesions consisted primarily of a fatty degeneration and necrosis of the smooth muscle fibers of the media, which later led to a crowding together of the elastic fibers into a relatively compact layer. The elastic fibers also underwent degeneration, and subsequently the degenerated tissue became extensively calcified. Calcification, however, occurred only in the animals that had received pituitary solution. Bailey felt that not too much emphasis should be laid on the importance of pituitary in the production of this change.

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2. Klotz, Oskar: *J. Exper. Med.* 7:633, 1905; 8:322, 1906; *Brit. M. J.* 2:1767, 1906.

3. Bailey, C. H.: *J. Exper. Med.* 25:109, 1917.

The results of the experiments reported in this paper confirm in the main those of Mollard and Regaud, Klotz and Bailey. In addition, some light is thrown on the development and the localization of the lesions in the aorta in the earlier stages of the intoxication and on the occurrence of calcification without simultaneous injections of pituitary or other substances.

#### EXPERIMENTS

Eleven rabbits were used in the experiments, as well as several others from the same lots which were kept untreated as controls. All of these were young animals, 6 months old or less. The diphtheria toxin was obtained from the Connaught Laboratories of the University of Toronto, through the kindness of Dr. P. J. Moloney. This toxin (no. 264) had a minimal lethal dose of approximately 1:800. The dilutions 1:200, 1:1,000 and 1:2,000 were used, depending on the dosage to be

#### *Details of the Experiments*

Rabbit	Weight, Gm.	Dose per Kilogram, Cc.	Number of Doses	Total Dose per Kilo- gram, Cc.	Duration of Experiment, Days	Lesions in Aorta
14	1,500	0.0050	1	0.0050	1½	Absent
15	2,000	0.0038	1	0.0038	4	Absent
16	2,000	0.0025	1	0.0025	2	Absent
17	2,100	0.0019	1	0.0019	5	Absent
6	1,400	0.00061	4	0.0024	8	Present
7	1,700	0.00059	5	0.0029	10	Present
24	1,970	0.00051	7	0.0036	14	Present
23	2,160	0.00046	6	0.0028	12	Present
27	1,390	0.00036	4	0.0014	12	Present
31	1,500	0.00025	15	0.0037	34	Absent
32	1,790	0.00013	16	0.0021	37 (Animal killed)	Absent

given; a dilution was chosen that gave for each dose a sufficiently large volume to be measurable with a moderate degree of accuracy. The diluted toxin in all cases was injected into the marginal vein of the ear.

In one group of animals, a single large dose was given, while in the remainder, smaller repeated doses were employed, the injections being separated by intervals of two or three days. In all cases but one (rabbit 32), the animals were allowed to die or else were killed only when obviously moribund.

The table gives the details of the experiments. The doses of the toxin have been reduced to terms of undiluted toxin per kilogram. The dose per kilogram was based on the weight of the animal on the day of the initial dose.

#### OBSERVATIONS

The animals all lost weight rapidly; one rabbit (no. 27) lost 510 Gm. in twelve days. All of the animals, with the exception of two that developed severe diarrhea (nos. 23 and 31), died without showing antemortem evidence of any infection or intercurrent disease.

Autopsy was carefully performed as soon as possible after death, and the aorta and the organs were preserved in 10 per cent formaldehyde. Frozen sections of the organs were stained with hematoxylin and sudan III, and paraffin sections were stained with hematoxylin and eosin.

Autopsy revealed no sign of intercurrent disease in any case, and the changes noted in the organs, both grossly and microscopically, were similar to those described by many previous investigators as attributable to the action of diphtheria toxin. These changes need not be described here. It might be noted, however, that in all animals that lived eight days or more, the adrenal glands were shrunk in appearance and reduced in size to approximately from one half to two thirds of the average size of the adrenals in the control animals. Frozen sections revealed the presence in the cortex of a greatly reduced quantity of lipid substance stainable with sudan III. The medullary portion showed no perceptible changes. In the control animals, autopsy revealed no macroscopic abnormalities of note, nor did microscopic examination reveal any changes in the organs and aortas.

As may be seen in the table, only five of the treated animals presented lesions in their aortas; in the aortas of the remainder no gross or microscopic changes were noted.

*Gross Examination of the Aortas.*—In the five aortas that showed lesions, these were macroscopically of a similar nature but of a varying degree of severity. The various stages in the development of the lesion may therefore be described without presenting individual protocols.

In the earliest stages of the degeneration, the adventitial surface of the aorta showed no abnormality. The intimal surface showed only a slight change, most marked in the arch and the thoracic portion and gradually diminishing to the vanishing point in the abdominal aorta. This change consisted of a very fine but distinct wrinkling of the intima, one set of these wrinkles running in the longitudinal direction and the other transversely. These fine striae gave the intima an appearance which might be likened to that of a very delicately woven fabric. The intimal surface was slightly rough to the touch, but the vessel was not perceptibly stiffened. There was a slight thinning of the wall of the aorta, but longitudinal stretching showed no obvious reduction in elasticity. In one animal (rabbit 6) the lesion was of about this degree of severity, but was confined to a narrow area longitudinally disposed on the anterior wall of the thoracic aorta and slightly toward the left side. It extended from the level of the first pair of intercostal arteries to the level of the diaphragm, forming a groovelike depression covered by a slightly puckered intima. Another small depressed area of similar appearance was present on the posterior wall in the descending part of the arch of this aorta.

With further advancement of the lesion, there was a diffuse progressive thinning out of the media accompanied by a wrinkling of the adventitial, as well as of the intimal, surface. The latter became rough and scaly to the touch, while the vessel as a whole was somewhat stiffened. Longitudinal stretching of the aorta revealed a marked loss of elasticity. The first parts of the large branches of the aorta also showed early changes of the same nature.

In the later stages, the wall of the aorta became extremely thin, and there was a disappearance of the wrinkles or puckerings on the intimal and adventitial surfaces, although the former remained rough and scaly to the touch. The aorta became greatly dilated, and transverse fissures appeared on the intimal surface, extending for a short distance into the media. They were spaced from 2 to 5 mm. apart throughout the length of the aorta. Sometimes the edges of such fissures were stripped up for 1 or 2 mm. on either side. These fissures could often be seen showing through the thinned out wall of the vessel, while the aorta lay in situ. Aneurysmal outpouchings of the aortic wall frequently occurred about the mouths of the large branches of the aorta, particularly about the great vessels arising from the arch, so that these took origin from the deepest part of the pouches. The first parts of the large vessels were themselves affected by a severe degeneration. In some specimens, shallow, dimple-like aneurysmal sacs were also present on the posterior wall of the thoracic portion of the aorta in the centers of the squares formed by successive pairs of the intercostal arteries. The aorta became stiffer and less elastic with increasing severity of the lesion. None of the specimens, however, in the gross showed conclusive evidence of calcification.

Throughout the process, the lesions tended to be most severe in the arch and thoracic portions of the aorta and less severe in the abdominal portion. At any given level, the damage appeared to be of equal severity around the whole circumference of the vessels, except in rabbit 6, in which the anterior segment of the thoracic portion of the aorta showed early changes, while the posterior segment appeared normal. Rabbit 6 showed the mildest change, and, in order of increasing severity, rabbits 7, 27, 24 and 23 showed more marked damage.

*Microscopic Examination of the Aortas.*—Sections of the aortas were taken at various levels. Frozen sections were stained with sudan III and hematoxylin and also with thionine. Sections of the aortas were also treated with 2 per cent nitric acid for twelve hours and subsequently cut, frozen and stained with sudan III. Paraffin sections were stained with hematoxylin and eosin, van Gieson's stain and Verhoeff's elastic tissue stain. The last two were sometimes used in combination. No special stain for calcium was used, and when in later paragraphs



the presence of calcium is indicated, the statement is based on the presence of aggregations of substances that showed a high affinity for hematoxylin.

Microscopic sections of the aortas of the five animals that presented changes in the gross specimens showed lesions that were of the same nature in all of them, but at various stages of progression. The sequence of events may therefore be described without the description of individual sections from each animal. Suffice it to say that microscopic examination fully confirmed the gross findings as to the position of the lesions and their relative degrees of severity.

The changes found in the aorta were, throughout, purely degenerative. No evidences of inflammatory processes were found in any of

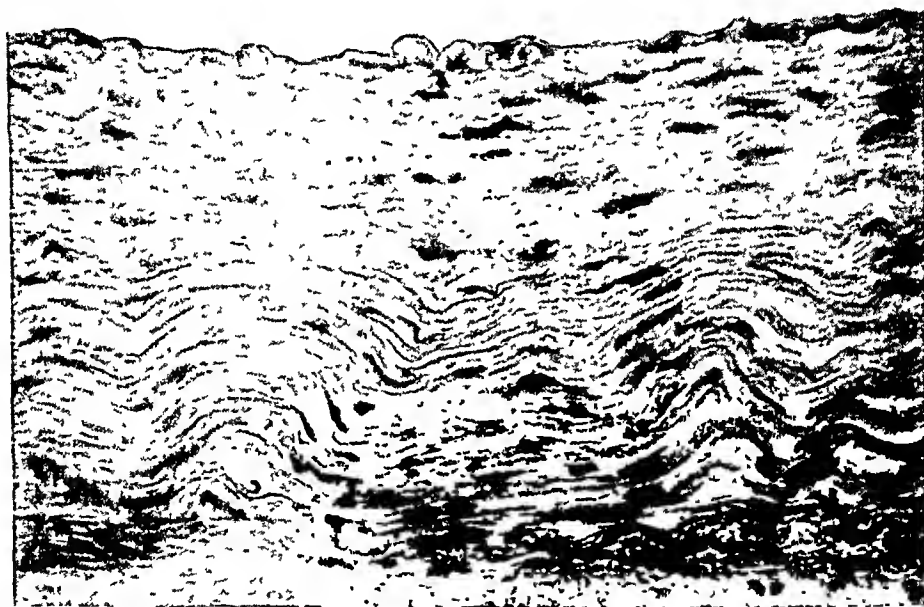


Fig. 1 (rabbit 7).—Thoracic aorta; hematoxylin and eosin;  $\times 400$ . The pyknotic nuclei of degenerated muscle fibers lie in the narrow spaces between the elastic lamellae in the middle third of the media. The clearer spaces produced by buckling of the elastic fibers contain the pale-staining flocculent debris of muscle degeneration.

the specimens. The media was primarily affected, changes being first noted about midway between the intima and the adventitia, in the middle third of the media. The degeneration in its later stages spread inward and outward, involving the whole thickness of the media in the specimens showing the most severe damage. However, the extreme peripheral portion of the media adjacent to the adventitia tended to be the longest spared. In none of the specimens did the intima show any proliferative change. It was affected only secondarily through alterations in the immediately underlying media.

The earliest change consisted of a localized degeneration of the muscle fibers in the middle of the media. The cytoplasm of these cells became slightly swollen and took on a cloudy flocculent appearance. With further swelling, this flocculent material became pale-staining, the cell outline became indistinct and hazy, and later it was replaced by an entirely irregular border. The nuclei of such fibers at first retained their staining properties, but a little later in the process they became shrunken and pyknotic, appearing as dark-staining, irregularly outlined, narrow, spindle-shaped bodies. Up to this point, the elastic fibers in such areas remained unchanged in their staining properties, but they appeared slightly swollen and their margins less distinct. Their normal, sharply kinked waviness was less apparent, giving place to gentler undulations in their course. Adjacent elastic laminae were separated

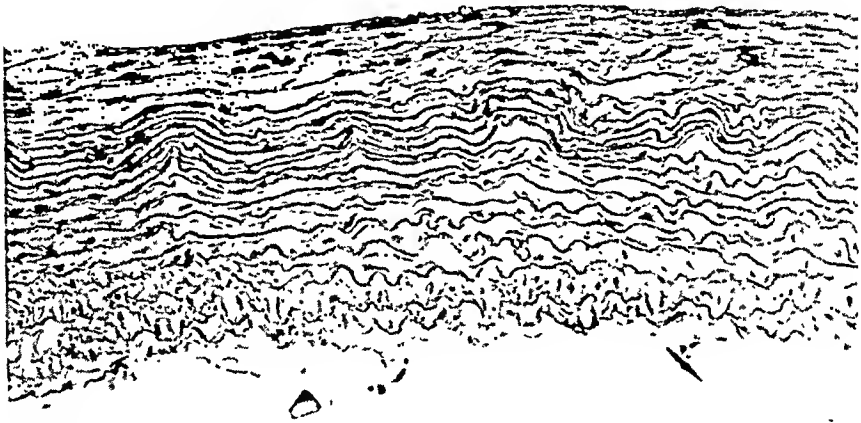


Fig. 2 (rabbit 7).—Thoracic aorta; frozen section stained with hematoxylin;  $\times 160$ . The middle third of the media shows the peculiar disposition of the elastic lamellae characteristic of the earlier stages of medial degeneration due to diphtheria toxin. A similar condition existed around the whole circumference of the aorta at this level.

farther from one another than is normally the case by the degeneration and swelling of the intercalated muscle fibers.

Early changes such as are indicated in this description were observed rather rarely, and occurred in areas adjacent to more severe lesions. In such situations, small localized areas in the middle third of the media were affected in this way. Lesions of greater severity were always found to involve considerable portions of the circumference of the vessel, or, more frequently, the whole circumference.

With more marked damage to the wall of the vessel, adjacent elastic laminae in the middle third of the media became pressed together, with a consequent condensation of the products of degeneration of the muscle fibers. The pyknotic nuclei still persisted. The elastic laminae in the

middle third of the media, thus compressed, came to lie close together and parallel to one another, with only a few long, sweeping undulations in their course. Here and there, their direction was suddenly altered by an obtuse angulation, which gave the impression of a certain stiffness or rigidity of these elastic fibers. It appeared as though they had been buckled by a postmortem spasm of the musculature still remaining in the inner and outer thirds of the media. However, there was as yet no evidence of calcification. At such angles the elastic laminae were sometimes separated from one another or from the more normal media on either side, leaving empty spaces or areas partly filled with pale-staining fragments of flocculent debris. The individual elastic fibers were even

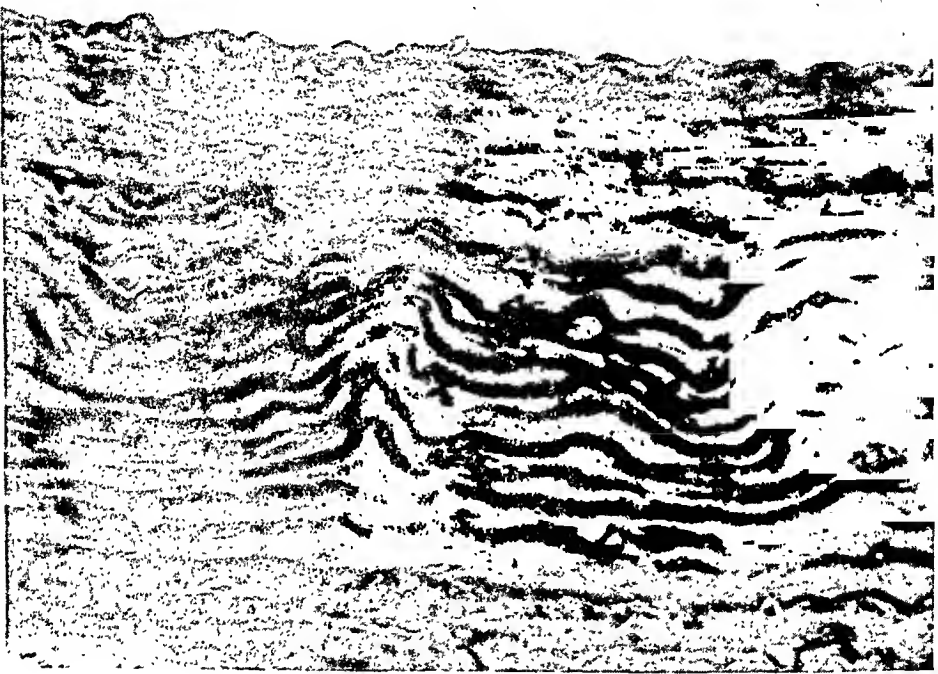


Fig. 3 (rabbit 7).—Thoracic aorta; Verhoeff's elastic tissue stain counterstained with van Gieson's picrofuchsin;  $\times 400$ . The elastic fibers in the middle third of the media are swollen in appearance. They lie close together in some areas, while in others buckling of the fibers has produced small spaces containing the pale-staining debris of muscle degeneration. Some of the elastic fibers show irregularity in their staining properties.

more swollen than before, and their outline was less distinct. They no longer stained normally with Verhoeff's elastic tissue stain, but showed at first a roughly granular appearance, while, later, entirely unstained portions of a single fiber were seen separated by portions that took the stain in an irregular fashion. No splitting, fraying or rupture of elastic fibers was noted.

In some specimens at this stage, beginning calcification was sometimes observed. It was first to be seen in flocculent debris of muscle

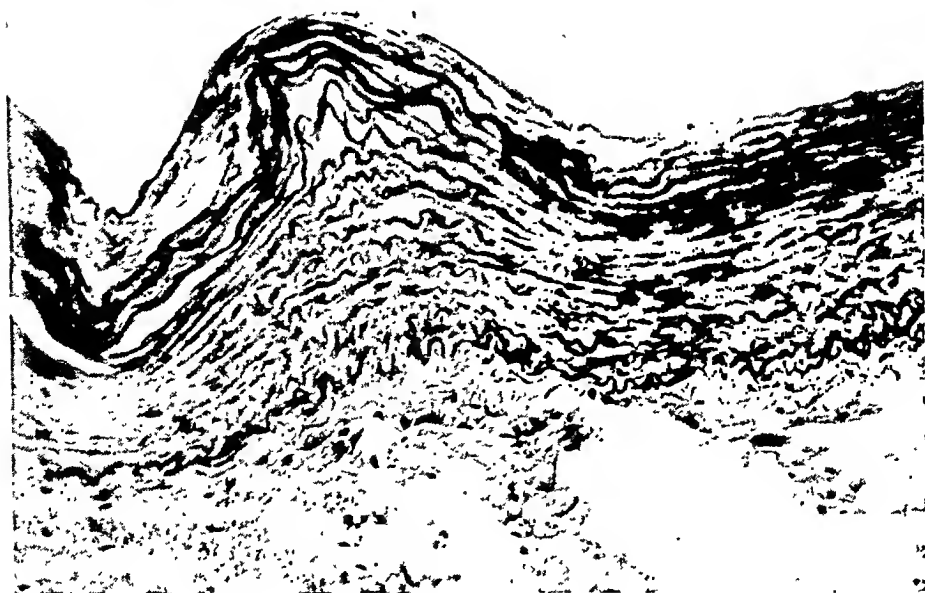


Fig. 4 (rabbit 27).—Thoracic aorta; Verhoeff's elastic tissue stain counterstained with van Gieson's picrofuchsin;  $\times 160$ . The degeneration has extended to the intimal surface. There is calcification in and around the innermost elastic fibers. A postmortem spasm of the musculature of the outer third of the media everted this aorta as soon as it was opened, so that its intimal surface faced outward.



Fig. 5 (rabbit 23).—Thoracic aorta; frozen section stained with hematoxylin;  $\times 400$ . The media is degenerated throughout its whole thickness. There is complete loss of undulations in the elastic lamellae. The calcified portions of the elastic fibers are separated by clear, uncalcified intervals. The calcification is most marked toward the intimal surface.

fibers as finely granular deposits. The deposits of calcium in the elastic fibers themselves were more homogeneous in appearance. These deposits occupied only short portions of the elastic fibers and were usually separated by quite wide intervals.

As the lesion progressed to severe degrees, the whole thickness of the media became involved by a process similar to that described. The media became much thinned out in consequence of the extremely close apposition of the swollen elastic laminae, which were separated only by the shrunk, pyknotic nuclei of the muscle fibers and a few remnants of flocculent débris. The degenerated elastic fibers took the elastic tissue stain only in their calcified portions, and were found lying as straight, parallel lines with no undulations. Calcification was marked in such specimens (rabbits 23 and 24), being most prominent in the elastic fibers, many of which showed calcification as far as they could be traced, with only an occasional short uncalcified interval. Degenerating



Fig. 6 (rabbit 24).—Thoracic aorta; Verhoeff's elastic tissue stain counterstained with van Gieson's picrofuchsin;  $\times 200$ . The media is degenerated throughout its whole thickness, and the elastic lamellae lie close together in parallel arrangement. Small uncalcified portions of elastic fibers do not take the Verhoeff stain. The calcification at this stage is most prominent in the elastic lamellae and most marked near the intimal surface. The rupture of the elastic fibers near the intimal surface is an artefact.

elastic fibers nearest the intimal surface tended to become calcified earliest and most completely. It was in areas that had reached this stage that transverse fissures were seen on the intimal surface in the gross specimen. Such splits extended through the calcified elastic fibers to a variable depth in the thinned out media.

In none of the specimens could any fatty substance be demonstrated by direct staining with sudan III. However, on treatment of the specimens with 2 per cent nitric acid and subsequent staining with sudan III as suggested by the studies of Klotz on calcareous degeneration, moderate quantities of fatty materials were found as extremely fine droplets distributed through the débris of muscle degeneration and

adherent to elastic fibers. The accumulations of fatty materials correspond closely with the areas in which calcification had been most marked in other preparations of the same part of the aorta. Fat was demonstrable in this way, however, only in those specimens that had shown calcification (rabbits 23, 24 and 27).

Staining with thionine and van Gieson's stain was suggested by the study of sections of so-called "spontaneous" lesions from the aortas of other rabbits. In such lesions, a substance could be found between elastic fibers that with the usual stains was, in appearance, not unlike the flocculent cloudy débris of degenerated muscle fibers described. However, in the "spontaneous" lesions, this material was stained bluish red by thionine and pink or red by van Gieson's stain, while the débris of degenerated muscle fibers in these experiments stained blue with thionine and yellow with van Gieson's stain.

#### COMMENT

Bailey has raised the question whether these widespread lesions are the result of the injections of diphtheria toxin or whether they are dependent on preexisting "spontaneous" lesions. I have had the opportunity of examining a number of the so-called "spontaneous" lesions of the aorta that occur not infrequently in older and larger rabbits. These lesions were almost without exception small, localized areas of medial degeneration and calcification, that appeared on the intimal surface, chiefly in the arch and the upper part of the thoracic aorta, as rounded, hard, whitish plaques, seldom more than 2 mm. in diameter. They differed from the lesions described here not only in their restricted distribution and distinct localization in very small areas, but also in the staining reaction of the pale flocculent material between the elastic fibers in and around the lesions. Moreover, a slight cellular infiltration was observed in the "spontaneous" lesions, while no evidence of inflammatory reaction in the media was found in the experiments. The possible predisposing influence of "spontaneous" lesions was removed as far as might be possible by the use of young rabbits 6 months of age or less. No "spontaneous" lesions were found in the controls nor any evidence of preexisting "spontaneous" lesions in the treated animals. I am therefore fully convinced that the lesions described as occurring in these experiments were due solely to the injections of diphtheria toxin.

The possibility has also been mentioned that the lesions in the aorta are not the result of a direct action of diphtheria toxin on the arterial wall, but are produced by an excessive secretion of epinephrine from the suprarenal bodies, which are known to be profoundly affected by diphtheria toxin. In the present experiments, the adrenal glands showed evidence of damage to the cortex, but the medulla was not

greatly altered in appearance. The results of experimental studies on the effect of diphtheria toxin on the secretion of epinephrine have been contradictory, probably owing to the differences in the experimental methods. However, the balance of opinion seems to be in favor of a diminution or even a complete suppression of epinephrine secretion, except with the most minute doses. The doses of toxin used in the present experiments were relatively large, and it therefore seems highly probable that the arterial lesions produced were due to the direct action of the diphtheria toxin on the media, rather than to an indirect effect through its action on the suprarenal medulla.

The action of the toxin in producing degeneration seems to be a remarkably rapid one. The shortest period after which lesions were found was eight days, and in Bailey's experiments seven days was the minimum. In these brief periods the lesions were quite well marked in at least some portions of the aorta, or else not present. Thus it would appear that there was a latent period of five or six days after which rapid degeneration took place. The rarity of early localized lesions in these experiments also speaks for a very rapid degeneration when once it is established.

The total dose of toxin was apparently not the determining factor in the production of the lesions, for, as may be seen from the table, the totals for some of the animals in which arterial lesions were found did not differ greatly from those from some animals in which the arteries were unaffected. The dosage evidently must be so regulated that a large quantity of unbound toxin is maintained in the circulation in such a way that the animal is not killed by it in less than seven or eight days. That a succession of small doses is more likely to meet these conditions is indicated by the work of de Croly,<sup>4</sup> who found that diphtheria toxin disappeared slowly from the blood stream in rabbits after intravenous injection, and that the rate of disappearance was proportional to its concentration in the blood. On the other hand, the doses may be so small that no arterial lesions are produced, even though treatment is continued over a much longer period. Thus, rabbit 27 showed less damage to the aorta than rabbit 23 or rabbit 24, while rabbits 31 and 32 showed no arterial lesions.

The localization of the lesions in the gross corresponded closely with that reported by previous investigators. The arch and thoracic portions of the aorta were earliest and most severely affected, and in these parts aneurysms were most frequently observed. As Klotz pointed out, the diaphragm may act as a dam throwing the greater part of the load of cardiac pulsations on the arch and the thoracic portions of the aorta, and therefore determining earlier fatigue in these areas. He

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4. de Croly, O.: *Arch. de pharmacod.* 3:61, 1897.

further showed, in his experiments on aortic lesions of this type produced in rabbits by the use of epinephrine, that blood pressure has an important bearing in the development of such lesions. The simultaneous administration of a drug that lowered the blood pressure, such as a nitroglycerin, while it did not prevent the production of lesions in the aorta, reduced their severity and extent.

Rabbit 6 showed a localization of the degenerative lesion in its early stages in the anterior segment of the thoracic aorta. Robertson<sup>5</sup> demonstrated anatomically that the blood supply to the media through the vasa vasorum is poorest in this area; the localization of the lesion in this animal may be explainable on the basis of poorer nutrition through the vasa vasorum. On the other hand, the localization of the lesion might be attributable to greater contractile activity of the aorta with correspondingly greater fatigue in its anterior thoracic portion, where it is not bound down to surrounding structures. It is possible also that both of these factors may play a rôle.

The localization of degeneration in its early stages to the middle third of the medial coat also speaks for the influence of poorer nutrition in determining the site of damage. This zone of the media is lacking in vasa vasorum and is dependent for its nutrition on diffusion of fluids either from the nearest vasa vasorum or from the intimal surface. It has thus a poorer nutritional supply than either the inner or the outer third of the media, which are accordingly involved latest by the degenerative process.

As might be expected, the actively contracting elements of the media, the muscle fibers, showed the earliest evidence of damage, while the elastic fibers were affected later. Bailey described a fatty degeneration of muscle fibers, while Klotz stated that the degeneration showed fatty changes in at least some stages of the process. In these experiments, no fat was found in any of the specimens on staining directly with sudan III. However, the demonstration of fat after decalcification indicated that the degeneration was, at least in its later stages, of a fatty nature. Calcification was apparently so rapid that the fat, as soon as it was released from the degenerating muscle fibers, became involved in the deposition of calcium and was therefore at no time stainable by direct methods.

The degeneration of the elastic fibers in the early stages would appear to have been of the nature of an alteration of the colloidal state of the materials composing them, as evidenced by the swelling, loss of distinct outline and slight reduction of elasticity, and yet without an obvious alteration of staining properties. Definite chemical changes probably took place later with the loss of normal staining properties

5. Robertson, H. F.: Arch. Path. 8:881, 1929.



and the beginning of calcification. These changes, however, never showed fat as an end-product. Even in decalcified specimens, no fat was demonstrated in elastic fibers, although fine droplets were frequently seen adherent to them. Before calcification had commenced, the fibers seemed to have acquired a certain stiffness or rigidity, obvious even in the gross specimen and also indicated microscopically by the peculiar buckling of degenerated but uncalcified elastic fibers. Bailey remarked on this phenomenon, which he also observed in his experiments. There was never any splitting or fraying of elastic fibers as described by McMeans<sup>6</sup> in human arteries, and rupture occurred only when calcification was advanced. The impression gained from examination of the sections was that the elastic fibers underwent a degeneration of extreme rapidity, second in this respect only to the degeneration of the muscle fibers.

Klotz pointed out the similarity between diphtheria lesions in the aorta and those produced by epinephrine, barium chloride and digitalin. One might also draw attention to the resemblance of these lesions in their general features to those produced by massive doses of vitamin D as first noted by Kreitmair and Moll<sup>7</sup> in 1928 and since observed by many others. All of these agents appear to be toxic in their action, and the slight differences in the lesions produced probably depend on differences in degree of toxicity and in the conditions of the experiments.

Klotz also indicated the resemblance of this type of lesion to the Moenckeberg type of arteriosclerosis in human arteries, and this observation was reiterated by Bailey. Comparison of the experimental lesions with those occurring in the Moenckeberg sclerosis in man may, at first sight, appear unreasonable because of the difference in the distribution of the lesions. The diphtheria lesions are localized chiefly to the upper half of the aorta, while the Moenckeberg type of arteriosclerosis in man is found in the peripheral arteries. The latter is best developed, however, in those arteries that might be presumed to have been called on for the greatest activity during the life of the individual. As has been pointed out in this paper, it is possible that the localization of the diphtheria lesions may also be distinctly conditioned by fatigue of the medial musculature and possibly also by poor nutrition. In the light of these considerations, the lesions are seen to be of a like nature as to some of their etiologic factors, as well as in their histologic appearance. To press the argument to its logical conclusion, one would suggest that the lesions of the Moenckeberg type in man are due to a nonspecific toxin, to the action of which certain peripheral arteries are predisposed by fatigue, with poor nutrition as a contributing factor.

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6. McMeans, J. W.: *J. M. Research* **32**:377, 1915.

7. Kreitmair, H., and Moll, T.: *München. med. Wchnschr.* **75**:637, 1928.

Direct application of these results to human diphtheria is rather precarious. One might suggest, however, the possibility of a relationship. Many investigators have questioned the statement that damage to the heart alone is sufficient to account for the circulatory collapse in rapidly fatal cases of diphtheria. Accordingly, damage to vasomotor centers has been invoked as a factor contributing to this collapse. It would seem at least possible from the present experiments that direct damage to peripheral arteries may also have a bearing on this phenomenon.

#### CONCLUSIONS

Successive intravenous injections of diphtheria toxin in suitable quantities produce in rabbits severe medial degeneration of the aorta and its large branches within from eight to fourteen days.

Damage to the arteries is probably the result of the direct toxic action of diphtheria toxin on the media.

The changes in the media are most marked in the arch and thoracic portion of the aorta, resulting in thinning of the arterial wall, dilatation and the formation of aneurysmal sacs. With the establishment of calcification, transverse fissures appear on the intimal surface.

The lesion commences in the middle third of the media, primarily as a cloudy swelling, degeneration and necrosis of muscle fibers. Fatty changes occur in the process at least in its later stages. Elastic fibers, slightly later, also undergo degeneration with the loss of elasticity and the development of stiffness and rigidity even before the appearance of calcification. Calcification is first seen as a finely granular deposit in the débris of degenerated muscle fibers, but later involves also the elastic fibers and becomes most prominent in them.

# A PRIMARY PULMONARY TUBERCLE APPEARING IN A PATIENT WITH ADVANCED HODGKIN'S DISEASE \*

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CHICAGO

The exciting cause and the identity of the important pathologico-anatomic structures of the clinical syndrome described by Hodgkin in 1832<sup>1</sup> remain in doubt. The controversy has narrowed itself down to a question of infection, malignancy and a rather indefinite group, each of which will be reviewed briefly in the order named.

The best evidence for the infectiousness of Hodgkin's disease is that the first manifestations are most commonly situated just outside the main portals of entry for infection. Furthermore, many infective agents have been found in the lesions, which nearly always resemble a chronic inflammation apparently spreading by infiltration, and not cellular metastasis. Sternberg,<sup>2</sup> Clark,<sup>3</sup> Reed<sup>4</sup> and others were of this opinion when the real controversy began at the beginning of the century. Reed expressed it well in the following statement:

Clinically there seems to be more evidence of its being of the nature of an infection. The course of the disease, though usually chronic, may be acute. We frequently have fever associated with other septic symptoms; analogous conditions are found in septicaemia and cachexias due to pyogenic organisms. The frequency with which the disease starts in the cervical region has suggested to many a probable source of infection in lesions of the mucous membranes or skin.

In addition to throat infections, various conditions have been ascribed as the cause of the disease, including whooping cough, exanthems, syphilis, infestation with animal parasites, leprosy, diphtheroid infections and, most important, infection with some form of the tubercle bacillus. Bunting's<sup>5</sup> work on diphtheroids and the works of Sternberg,<sup>2</sup> Fraenkel and Much<sup>6</sup> and L'Esperance<sup>7</sup> on the tubercle bacillus are outstandingly representative of reports on infection.

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\* From the Research Laboratories of the Municipal Tuberculosis Sanitarium.

1. Hodgkin, T.: *Tr. Med.-Chir. Soc., Edinburgh* **17**:68, 1832.

2. Sternberg, C.: *Ztschr. f. Heilk.* **19**:21, 1898.

3. Clarke, J. M.: *Brit. M. J.* **2**:701, 1901.

4. Reed, D. M.: *Johns Hopkins Hosp. Rep.* **10**:133, 1902.

5. Bunting, C. H.: *Bull. Johns Hopkins Hosp.* **26**:179, 1915.

6. Fraenkel, E., and Much, H.: *München. med. Wchnschr.* **56**:685, 1910.

7. L'Esperance, E. S.: *J. Immunol.* **16**:27, 1929.

On the other hand, there is no case of authentic Hodgkin's disease in which the condition, as such, has been transmitted to another host, and, what is more significant, no recoveries have been recorded in medical literature. These facts speak strongly against infection and for malignancy. Among those who hold the theory that the disease is malignant are Gibbons,<sup>8</sup> Mallory,<sup>9</sup> Warthin,<sup>10</sup> MacCarty<sup>11</sup> and Medlar.<sup>12</sup>

There is another smaller group who consider the disease neither infectious nor malignant, but an active proliferation of all hemato-poietic elements. Lubarsch<sup>13</sup> and Symmers<sup>14</sup> are representative of this group.

The problem seems, therefore, to be to reconcile these dominant and apparently paradoxical views. The purpose in this report is to aid in this attempt.

It would be inappropriate to give here any extensive history of this subject. For such a review, reference may be made, among others, to the works of Sternberg,<sup>2</sup> Reed,<sup>4</sup> Fabian,<sup>15</sup> Ziegler,<sup>16</sup> Hirschfeld,<sup>17</sup> Herxheimer<sup>18</sup> and Lemon,<sup>19</sup> and to the relatively recent review of Simonds.<sup>20</sup> The more important reports dealing with the tubercle bacillus, however, will be mentioned.

While the controversy as to the nature of the process has existed for several decades, it was Sternberg<sup>2</sup> in 1899 who opened up the subject by attempting to separate the disease from other swellings of lymph nodes, "as a peculiar form of tuberculosis." Although by 1909 he had become less dogmatic, in 1923 he felt that evidence was beginning again to support his first contention. The work of Fraenkel and Much<sup>6</sup> in 1910 and 1923, on finding "granular forms of tubercle bacilli" in twelve of thirteen cases of Hodgkin's disease, seemed like strong corroborative evidence. Hirschfeld,<sup>17</sup> Sisto,<sup>21</sup> Lichtenstein<sup>22</sup> and Ewing<sup>23</sup>

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8. Gibbons, H. W.: *Am. J. M. Sc.* **132**:692, 1906.

9. Mallory, F. B.: *Principles of Pathologic Histology*, ed. 1, Philadelphia, W. B. Saunders Company, 1914, p. 326.

10. Warthin, A. S.: *Ann. Surg.* **93**:153, 1931.

11. MacCarty, W. C.: *J. Cancer Research* **14**:394, 1930.

12. Medlar, E. M.: *Am. J. Path.* **7**:499, 1931.

13. Lubarsch, O.: *Berl. klin. Wchnschr.* **55**:708, 1918.

14. Symmers, D.: *Am. J. M. Sc.* **167**:157 and 313, 1924.

15. Fabian, E.: *Centralbl. f. allg. Path. u. path. Anat.* **22**:145, 1911.

16. Ziegler, K.: *Die Hodgkinsche Krankheit*, Jena, Gustav Fischer, 1911.

17. Hirschfeld, H.: *Folia haemat.* **15**:183, 1913.

18. Herxheimer, G.: *Beitr. z. klin. d. Infektionskr.* **2**:349, 1914.

19. Lemon, W. S.: *Am. J. M. Sc.* **167**:178, 1924.

20. Simonds, J. P.: *Arch. Path.* **1**:394, 1926.

21. Sisto, P.: *Policlinico (sez. med.)* **26**:209, 1919.

22. Lichtenstein, A.: *Frankfurt. Ztschr. f. Path.* **24**:529, 1921.

23. Ewing, J.: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1919.

produced tuberculosis by inoculating material from lesions of Hodgkin's disease into guinea-pigs. It was mostly of a benign type. Ewing, however, later considered the two conditions to be frequently associated, but felt that the whole problem was still one of confusion. Ziegler<sup>16</sup> reported that 20 per cent of the cases of Hodgkin's disease showed tubercle bacilli, and that 10 per cent would cause infection in guinea-pigs. Reed,<sup>4</sup> Simmons,<sup>24</sup> Longcope<sup>25</sup> and Askanazy,<sup>26</sup> however, failed to produce the disease in guinea-pigs, and the latter two also failed to produce it in monkeys. Lemon<sup>19</sup> analyzed a group of cases and found that tuberculosis was less common than in the ordinary population. Herxheimer<sup>18</sup> considered the cause a mutant form of the human tubercle bacillus, while Baumgarten<sup>27</sup> suggested an altered form. Benda,<sup>28</sup> Sisto,<sup>21</sup> Lichtenstein<sup>22</sup> and Sticker and Löwenstein<sup>29</sup> reported that the characteristic changes of Hodgkin's disease followed the first inoculations in guinea-pigs, but that typical tuberculosis developed on passage. Kawatsure<sup>30</sup> also reported in favor of the tuberculous origin. I<sup>31</sup> reported the finding of the Sternberg-Reed type of giant cells in a guinea-pig inoculated with the Berkefeld filtrate of tuberculous material, but have been unable to repeat it. I<sup>32</sup> also produced a peculiar tuberculosis in guinea-pigs from four of five specimens of tissue showing Hodgkin's disease. It was my opinion (but not now) that the process looked more like an infection by an attenuated or mutant form of tubercle bacillus in a special type of host. Sticker and Löwenstein<sup>29</sup> reported the finding of the bovine type of tubercle bacillus, and L'Esperance,<sup>7</sup> after producing tuberculosis in chickens, thought the avian type might be the cause. Twort,<sup>33</sup> in a series of forty cases, was unable to confirm this or, in fact, to add anything definite, and I was unable to produce tuberculosis in chickens from one of my strains that grew and looked like the avian strain. Although the negative reports are many (Simmons, Reed, Longcope, Askanazy), it is generally conceded now that tubercle bacilli may be present in lesions of Hodgkin's disease, but as secondary invaders (Reed, Longcope, Lemon). Weber<sup>34</sup> thought that the lesions of Hodgkin's disease afforded good soil for the growth of tubercle bacilli. Those who have tried to reconcile the theory that

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24. Simmons, C. C.: *J. M. Research* **9**:378, 1903.

25. Longcope, W. T.: *Bull. Ayer Clin. Lab., Pennsylvania Hosp.* **1**:1, 1903.

26. Askanazy, M.: *Verhandl. d. deutsch. path. Gesellsch.* **15**:86, 1912.

27. Baumgarten, P.: *München. med. Wchnschr.* **61**:1545, 1914.

28. Benda, C.: *Verhandl. d. deutsch. path. Gesellsch.* **7**:123, 1904.

29. Sticker, A., and Löwenstein, E.: *Centralbl. f. Bakt.* **1**, O. **55**:267, 1910.

30. Kawatsure, S.: *Frankfurt. Ztschr. f. Path.* **31**:450, 1925.

31. Sweany, H. C.: *Am. Rev. Tuberc.* **17**:77, 1928.

32. Sweany, H. C.: *Tr. Chicago Path. Soc.* **22**:66, 1928.

33. Twort, C. C.: *J. Path. & Bact.* **33**:539, 1930.

34. Weber, F. P.: *St. Barth. Hosp. Rep.* **43**:81, 1908.

Hodgkin's disease springs from tuberculosis by means of tuberculin reactions have obtained only negative results (Simonds). Reed made tuberculin tests in five cases, and in all the results were negative.

The following report deals with a case of advanced Hodgkin's disease in which it was possible to demonstrate one of the earliest primary tuberculous complexes yet recorded. The study presents several problems of deep scientific import, the most important of which is that more than any proof heretofore offered it seems to indicate that Hodgkin's disease may exist without any form of tuberculosis being present.

#### REPORT OF CASE

S. M., a 6 year old white girl, came to the sanatorium complaining of glandular swelling of the right side of the neck lasting ten months. The family history was negative; there was no known contact with tuberculosis; home conditions were fair; there was no deprivation or dissipation. She had had chickenpox and whooping cough.

The patient was well until Dec. 25, 1929, when her mother noticed a swelling of the right side of her neck. This swelling was never painful. For a time it grew larger. Subsequently, the mother thought the swelling had decreased. The patient weighed 41 pounds (18.6 Kg.) on admission; she lost no weight afterward. No fever or cough was observed. The general development was poor. There was slight anemia. The general condition was fair. The teeth were carious, with several decayed stumps. The anterior cervical glands were enlarged, especially those on the right side. The colon was palpable. The tonsils were slightly enlarged. A vaccine scar was noted. The chest expansion was somewhat decreased on the right; the resonance was slightly impaired at the apexes posteriorly; there were no adventitious sounds. From these findings a diagnosis of tuberculous cervical lymphadenitis (nonsuppurative) with secondary anemia was made. The report of the roentgen examination by Dr. Carrol E. Cook follows. "The apices are hazy; the diaphragms regular; the costophrenic angles clear. The cardiac shadow is enlarged both to the right and to the left. There is an increase in the usual lung-root markings, on both sides, without parenchymal extension. The findings are those of a hilus process with a cardiac complication, most probably tuberculous."

The last monthly examination of the patient by Dr. Hurwitz, Feb. 23, 1931, revealed that the patient felt, in general, fair and had moderate appetite and satisfactory digestion, with regular movements of the bowels, and that her strength and general condition were fair. There was anemia with slight icterus. The lungs were essentially normal. The heart was enlarged, with a hemic murmur at the apex and over the pulmonary artery. The liver and spleen could be palpated down to the umbilicus. The lymph nodes on the right side of the neck were enlarging. The general condition was worse.

The rises in temperature during the first month occurred in from ten to twelve day cycles, the fever lasting from three to four days, and gradually rising to a continuous curve ranging from 101 to 104 F. A tuberculin test was not made because of the elevations of temperature.

The sputum and the urine were always negative for tubercle bacilli. The blood on Nov. 13, 1930, revealed: erythrocytes, 5,110,000; leukocytes, 12,900; hemoglobin 58 per cent. On Feb. 10, 1931, it showed: erythrocytes, 2,220,000; leukocytes, 11,800; hemoglobin, 25 per cent; neutrophils (polymorphonuclears), 74.5 per cent

and (myelocytes) 1.5 per cent; eosinophils, 1 per cent; basophils, 0.5 per cent; transitionals, 11.5 per cent; small lymphocytes, 4 per cent; large lymphocytes, 2.5 per cent, and large mononuclears, 4.5 per cent. The results of the Kahn and Wassermann tests on Nov. 19, 1930, were negative. Examination of a lymph node on Feb. 24, 1931, showed: the capsule intact and grayish white, the cut section uniformly gray, the microscopic architecture of the gland entirely obscured by diffuse hyperplasia of reticulum cells and lymphoid cells, and the cells large, with vesicular nuclei and eosinophilic nucleoli. There were no Langhans' giant cells. The diagnosis was Hodgkin's disease.

Death occurred on March 1, 1931, and autopsy was performed on the following day.

*Autopsy.*—The body was that of a fairly well nourished, white girl about 7 years of age, 4 feet, 9 inches (144.8 cm.) in length, and weighing about 40 pounds (18.1 Kg.). The skin and mucosae were very pale. The cervical lymph nodes were slightly enlarged. There was a recently sutured operative incision, 4 cm. long, over the right carotid triangle. The level of the abdomen was one fingerbreadth above that of the chest. No axillary or inguinal glands were palpable. The mid-line fat was 2 mm. thick. The peribiliary, peripancreatic and periaortic glands were prominently enlarged to a mass 10 by 10 by 5 cm. The liver showed two white, slightly elevated areas, and the spleen many similar, large, irregular nodes.

The pleural cavities were entirely free from adhesions. The pericardium contained about 30 cc. of clear, pale fluid.

The heart weighed 170 Gm. The left ventricle measured 10 mm. in thickness; the right ventricle, 4 mm. The myocardium was reddish brown and moderately firm. The endocardium was smooth. The coronary arteries were thin-walled and patent. The aortic intima was smooth throughout.

The liver weighed 980 Gm. It was smooth, reddish brown and moderately firm. At the lower border of the right lobe and along the upper border of the left were irregular, firm, minimally elevated nodes up to 3 by 4 cm. in diameter, yellow-gray, with brownish, isolated and enmeshed streaks. These nodes were rather sharply demarcated from the surrounding tissue, which was yellow-brown and moderately firm, the markings slightly obscured. The cut section revealed two similar nodes, deep in the hepatic parenchyma.

The spleen weighed 110 Gm. It was moderately enlarged, roughly nodular, purplish red and firm. Externally, the nodes ranged from 1 to 3 cm. in diameter, were roughly spherical and yellow-gray, with prominent purplish mottling. The cut section revealed a similar structure with similar nodes embedded in the splenic substance. The nodes were in the main isolated. The remaining tissue was deep red and firm.

The thymus could barely be identified.

The kidneys together weighed 200 Gm. They were red-brown, smooth and moderately firm. The capsule stripped easily, leaving a smooth red-brown surface. The cut section revealed distinct markings.

The suprarenal glands weighed 8 Gm. Their lipid content was diminished.

The intestines, urinary bladder, tubes, ovaries and uterus appeared normal. The pancreas weighed 60 Gm.; it was pinkish gray and lobulated.

The cervical lymph nodes were discrete, soft, yellow-gray, and enlarged only to a diameter of 6 mm. The mediastinal lymph nodes were not enlarged. The periaortic, peripancreatic and peribiliary nodes were fused into a loose mass. The individual nodes reached a diameter of 3 cm. They were firm, pale and yellow-gray. On cut section, the node boundaries were still visible. The cut surface was glistening, streaked gray and homogeneous. One node contained an irregular area,

1 cm. by 2 cm., of soft pultaceous material, surrounded by a zone of golden pigment, 2 mm. wide. In this caseous mass were several small foci of calcification.

The lungs had bilateral, basal bronchopneumonia involving almost completely the lower lobes and the bases of the other three lobes. At the apex of the right lower lobe, posteriorly, there was a primary tubercle measuring 3 mm. across. There was an early glandular complex toward the hilus involving the proximal border of the gland, also a smaller pleural complex extending to the hilar nodes by way of the pleural lymphatic vessels.

*Anatomic Diagnosis.*—Small, early, primary tuberculous lesion at the apex of the right lower lobe, with a lymphatic complex by way of the bronchial and



Fig 1.—A posterior view of the lungs, arrows indicating the primary lesions. (About half the natural size)

pleural lymphatic vessels; bronchopneumonia of both lower lobes; Hodgkin's disease of the cervical, periaortic, peripancreatic and peribiliary lymph nodes, spleen and liver; parenchymatous degeneration of the kidneys; diminished lipoid content of the suprarenal glands; moderate enlargement of the cervical and mesenteric glands; recent operative incision of the neck; marked anemia, and involution of the thymus.

*Microscopic Examination*—There were no gross or microscopic changes outside the lungs to indicate foci of tuberculosis. The local pulmonary lesion situated just beneath the pleura measured 3 mm. across the caseous portion, with a ring of inflammatory tissue from 2 to 3 mm. wide around it. There were a few smaller foci around the larger one, but they did not show caseation. The principal lesion revealed a slight excavation in what appeared to be a terminal bronchiole with a



slight zone of caseation around it undergoing sloughing. The main body of the lesion consisted of early caseation of the bronchopneumonic focus, which is considered specific for a primary lesion by Ranke,<sup>35</sup> Pagel and Henke,<sup>36</sup> Schulze,<sup>37</sup> Huebschmann<sup>38</sup> and others.

From this local site the infection traveled toward the hilus by two routes: (1) the pleural lymphatic vessels and (2) the bronchial lymphatic vessels. The pleural lymphatic vessels could be followed as small, white, threadlike elevations toward the hilus of the lobe, where they perhaps joined the bronchial vessels. The bronchial lymphatic route could be followed from one lymph node to the other, the side next to the parenchymal lesion showing the oldest part of the lesion. Microscopically, there was a typical tuberculous infiltration with Langhans' giant cells, "epithelioid" cells and all the other characteristics of a tuberculous process. No evidence could be found of atypical cells. No Sternberg-Reed cells were found in the lungs, pleura or their lymphatic vessels.

The lesions in the cervical and peripancreatic lymph nodes and the lesions in the spleen and liver were typical of Hodgkin's disease. In fact, an unusual number



Fig. 2—A cross-section of the primary lesion Hematoxylin and eosin;  $\times 85$ .

of specific giant cells were present. The ages of the various lesions varied, as indicated by the type of cell and the amount of fibrosis. There were no processes that could be mistaken for tuberculous inflammation, although tubercle bacilli were present on inoculation into animals. A more complete description of these lesions will be given in a subsequent article, in which an attempt will be made to trace their evolution.

*Inoculation Experiments.*—On March 2, 1931, the diseased lymph nodes and splenic nodules were macerated separately and prepared each for a series of inoculations, to see whether or not any unusual infectious agent was present.

35. Ranke, K. E.: *Deutsches Arch f. klin Med.* **119**:123 and 129, 1916

36. Pagel, W., and Henke, R., in Henke and Lubarsch: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 3, pt. 2, p. 190.

37. Schulze, E.: *Beitr. z. klin d. Tuberk.* **68**:216, 1928.

38. Huebschmann, P.: *Pathologische Anatomie der Tuberkulose und ihre Grenzgebiete in Einzeldarst*, Berlin, Julius Springer, 1928, p. 158

Guinea-pig Y90 was inoculated subcutaneously with macerated lymph nodes. When killed, April 18, 1931, it presented atypical tuberculosis. The macerated spleen of Y90 was remoculated into six guinea-pigs and four chickens on April 18, 1931. In all the guinea-pigs typical tuberculosis developed. The chickens were inoculated intravenously, but all were normal when killed on June 26, sixty-nine days later.

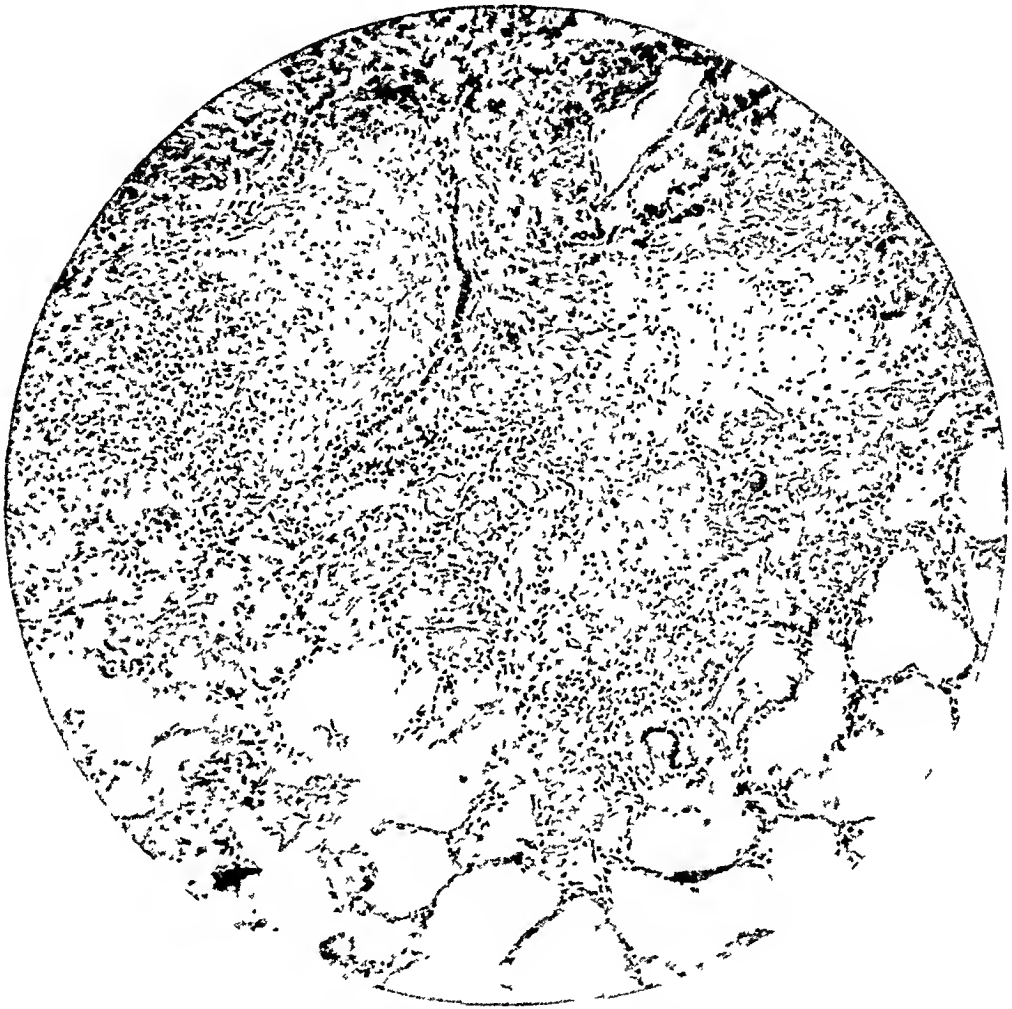


Fig. 3—A portion of figure 2 (marked by arrow) showing typical tuberculous inflammation with Langhans' giant cells. Hematoxylin and eosin;  $\times 100$ .

Guinea-pig Y92, the companion of Y90, was inoculated subcutaneously on March 2, 1931, and killed on April 20, and found to be tuberculous. Rabbit Y93, inoculated intravenously, died of "shock" immediately. Rabbit Y94, also inoculated, was killed on April 27, and found to be normal. Two more animals were inoculated subcutaneously at the same time. One died on April 28, of pneumonia. The other was killed on the same date and found to be normal. Two rats inoculated on the same date were killed on April 28. No changes were found. Two chickens were inoculated intravenously on the same date. One died in six days of "pneumonia." The other was killed on April 27, and was found apparently normal.

Berkefeld filtrates, through three separate "N" filters, of macerated nodules from the diseased spleen were inoculated into guinea-pigs on March 3, 1931. Y97 died on September 5, and was found normal. Y98 died on March 26. Pneumonia was shown, but the animal's lungs were too putrefied for accurate study. Y99 was killed on April 20. The inguinal lymph nodes were slightly enlarged, but all else was normal.

Berkefeld filtrates, through "N" filters, of macerated nodules from diseased lymph glands were inoculated into guinea-pigs. Y100 and K1 were killed on April

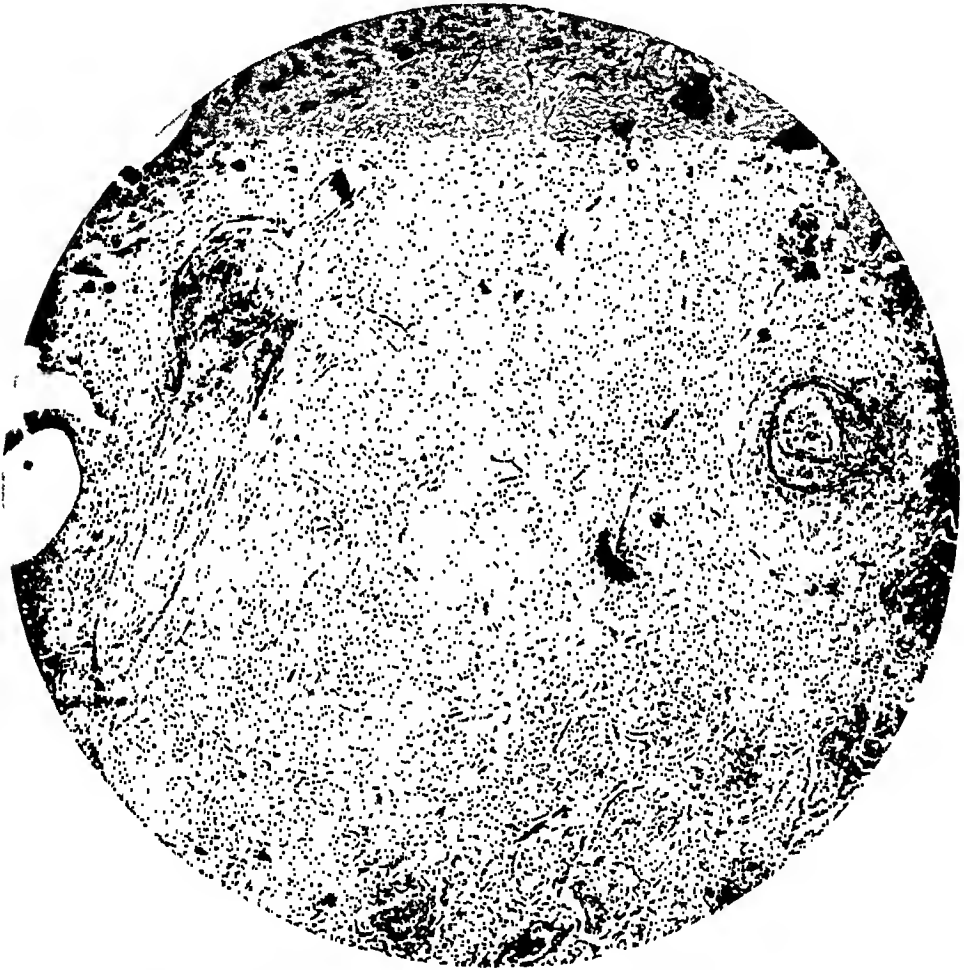


Fig. 4.—The center of the caseous tubercle shown in figure 2, disclosing elastic fibers in the blood vessels and alveoli. Weigert's stain;  $\times 85$ .

20, 1931, K2 on June 26, K3 on April 28, K4 on June 26, and K5 on December 15, and all were found normal.

Chicken 1 was inoculated on March 3, 1931, with "mixed filtrates"; it was killed on April 29, and found to be practically normal. Chicken 2 was killed on April 28, and a few small foci of fatty change were found in the liver. Reinoculation was made, April 28, into two guinea-pigs and two chickens to see whether these slight changes might be significant. Guinea-pigs K76 and K77, inoculated subcutaneously, were killed on June 26 and found normal. Chicken A was killed on June 26, and found normal. Chicken B was killed on September 16, and found normal.

It was concluded, therefore, that only human tubercle bacilli were present in the patient, and that no filtrable forms could be demonstrated.

## COMMENT

There seems to be clear evidence that a primary tuberculous infection occurred in the lungs of this patient fully a year after the Hodgkin's disease had developed and at about the time a biopsy had established the diagnosis of Hodgkin's disease in a distant organ. From the point of view of age, the tuberculous lesion appeared to be

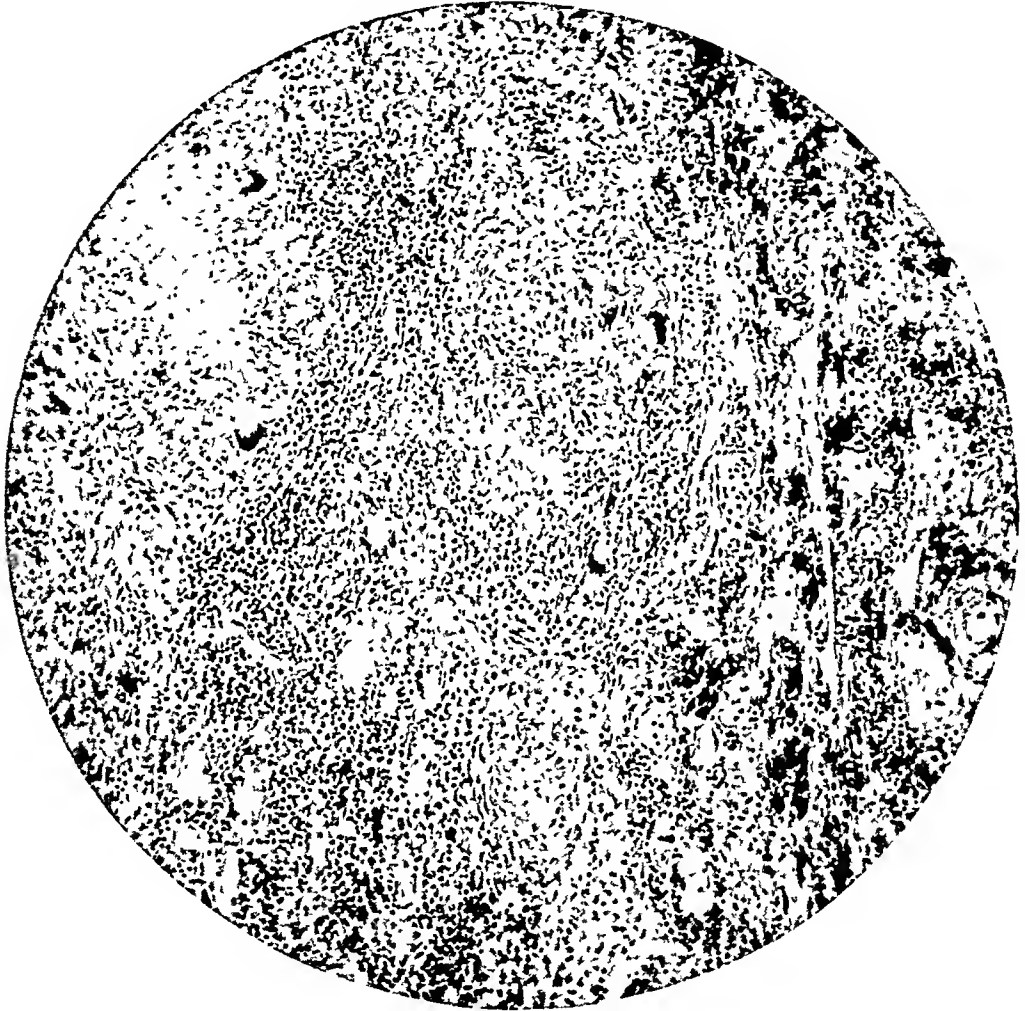


Fig. 5.—A caseous hilar lymph node showing typical tuberculous inflammation with Langhans' giant cells;  $\times 85$ .

similar to those in animals at from four to six weeks after infection. The source of the infection here is entirely immaterial, although there was a possibility of contact with other children who had open tuberculosis.

The finding of tubercle bacilli in the abdominal lymph nodes that showed Hodgkin's disease can easily be explained. The hematogenous phase of a primary lesion the size of this one may not cause extra-

pulmonary metastases, although bacilli certainly are disseminated in varying numbers. There is perhaps a quantitative relation between the number of bacilli in the blood and tissues and the production of the specific changes of tubercle formation.

As will be seen from the experiments, I was unable to show the presence of any type of tubercle bacillus but the human, and the presence of this I believe resulted from an exogenous infection. This focus, no doubt, produced a hematogenous dissemination to other organs including the nodes and the spleen in which Hodgkin's disease was shown and where the bacilli were recovered. I was unable to confirm



Fig. 6.—Cross sections of spleen, liver and peripancreatic lymph nodes, showing characteristic lesions of Hodgkin's disease. (About half of the natural size.)

L'Esperance's findings of avian or even of avian-like tubercle bacilli in this particular instance. In a former study,<sup>32</sup> I did find atypical acid-fast bacilli in the lesions of Hodgkin's disease, but they were like the avian bacillus only in a few physical ways. The conclusion that such forms are avian on the sole ground that they may kill chickens and may resemble the avian form is not justified. They must be identical in every way, for, as suggested by Branch,<sup>39</sup> if they can lose virulence, why can not the human strain? The strains that I isolated, although smooth, moist, rapid growers, did not kill chickens. Then, there was not one strain but many, appearing much like a "shower of

39. Branch, A. *Arch Path.* 12:253, 1931.

mutants." Such atypical forms of bacilli are not confined to Hodgkin's disease, in my experience, but occur commonly in other processes recognized as tuberculous, and occasionally secondarily in pulmonary cancers and processes not related to tuberculosis. This observation is perhaps similar to that reported by Rabinowitsch-Kempner.<sup>40</sup> So far as my experience is concerned, therefore, the avian tubercle bacillus is ruled out.

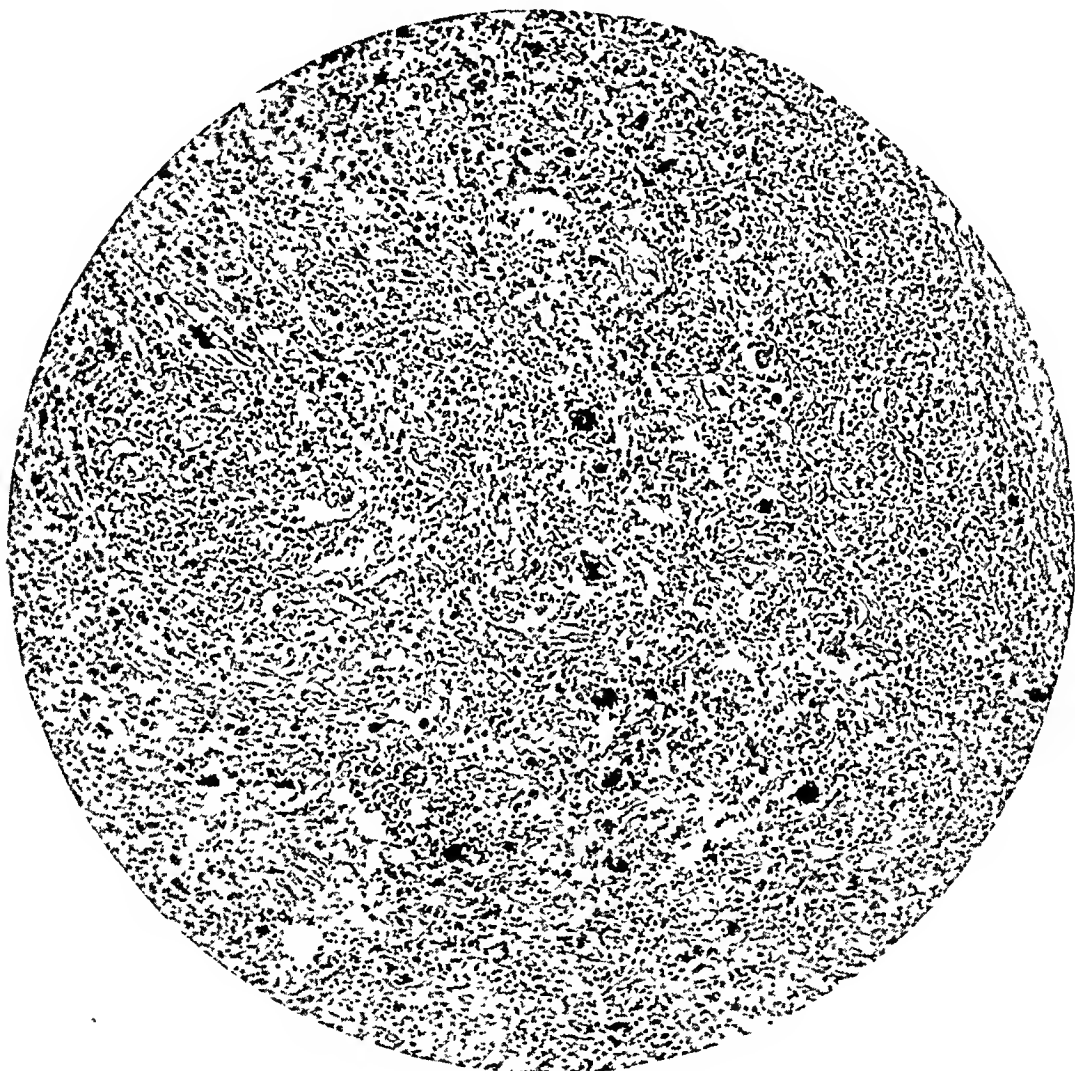


Fig. 7.—A section of the lymph node shown in figure 6, disclosing typical Sternberg-Reed giant cells, lymphocyte-like cells and fibrous tissue;  $\times 85$ .

The theory that an atypical tubercle bacillus may cause the disease was also not supported, nor were any diphtheroid or filtrable forms found.

It is unlikely, therefore, that tuberculous infection was present except in the pulmonary focus.

40. Rabinowitsch-Kempner, L.: *Am. Rev. Tuberc.* **15**:225, 1927.

The only question is whether it was primary or secondary. The main points of difference between a primary and a secondary tubercle are that in the former the caseous bronchopneumonia evolves much like an ordinary pneumonia except that it goes on to caseation, calcification and sometimes excavation, but there always remains the basic tissue framework (in the unexcavated part), which may be demonstrated by suitable staining. Weigert's stain of a cross-section of this tubercle showed a complete framework of the alveoli and blood vessels up to the edge of excavation. Around the periphery, the alveoli were plugged with fibrin containing scattering polymorphonuclears, lymphocytes and "epithelioid" cells. There were many plugs of almost pure fibrin. Epithelioid cells were numerous in certain places, but there was no sign of a cellular or fibrous capsule. In contrast, a secondary tubercle has an avascular center with homogeneous, obliterative caseation necrosis or a secondary bronchopneumonia that is diffuse, extensive and irregular, with no signs of encapsulation and little or no glandular complex. An isolated lesion the size of the one described, therefore, seems to be primary.

In order to establish the contrary, it would be necessary to assume that the infection of the lymph node progressed for over a year without producing local specific lesions and without sending out any blood-borne metastases to the lung, but that just prior to death one single focus became lodged beneath the pleura and produced a typical primary tuberculous lesion. If such an origin were to occur by some subtle transmutation, one should have evidence of it in the pulmonary parenchyma or in the hilar lymph nodes at an earlier date; the foci would be multiple; they would tend to be located in the upper parts of the lung, and they would be of a secondary type and not typically primary.

Although this does not disprove the theory that Hodgkin's disease in other instances is infectious in origin, it practically rules out tuberculosis as a cause in this case, and with it the foundation stones of the whole infection theory are severely shaken. Of course, there still may be some unknown bacterial agent, such as, for example, a filtrable virus or a toxic agent, as suggested by Benda,<sup>28</sup> but the probability seems a great deal less. If any specific infectious agent is the cause, the tubercle bacillus and its mutants seem to have the bulk of support. Now it seems that one must look on the tubercle bacillus either as a nonspecific irritant or as a secondary invader, for low grade tubercle bacilli may be present without producing a tubercle. On the other hand, toxic material from carious teeth and tonsils may be important factors, indirectly if not directly.

With these facts before me, I am compelled to view Hodgkin's disease more in another light—perhaps that of a malignant process. As

most malignant diseases are initiated by an irritant, it is not an unreasonable theory to suppose that the variety of agents found represent at most a form of irritant that is of such a low grade of virulence that only a toxic reaction is produced without any constant specific changes. Whether this irritation leads to a malignant condition, only time and patient work will reveal.

The most difficult obstacle for those adhering to the infection theory to surmount is that Hodgkin's disease is always fatal. Few infections are. In practically every infection there are some recoveries. It is only sound biology to expect this.

Turning from the infection theories, there seems to be a strong argument for the theory that a malignant development is the cause of Hodgkin's disease and of various other fatal diseases of similar origin. Some of these views I shall mention, leaving out any attempt to reconcile the views of that group which considers it a "hemopoietic hyperplasia," for lack of definite information.

In spite of the allurements of the theory recently advocated by Medlar,<sup>41</sup> suggesting an origin in the bone marrow, there are still conditions that this theory does not satisfy, most important of which is that the disease manifests itself most frequently near a portal of entry and in the lymph nodes. If the disease were first a process of the bone marrow, there should be more gross marrow changes similar to those of multiple myeloma, and the metastases to the lymph nodes should involve the lymph glandular system at random and not just those near the great portals of entry. Until these changes can be proved to originate in the marrow, it seems easier to believe the process to be of either lymph node or reticulo-endothelial origin. When one thinks of the wide dispersion that may result from changes in the simple cells such as lymphocytes, "monocytes," "histiocytes," etc., as shown by Maximow<sup>41</sup> and others, can one afford to be too dogmatic, for example, about a still earlier although hypothetical type of cell of the mesenchyme? Mallory's<sup>9</sup> idea of a lymphoblastic origin seems to be more tenable, although there is yet no agreement as to what constitutes a lymphoblast. At any rate, the general understanding is that it is a "blast" cell of the lymph node germinal centers, which may resemble either the lymphoid or the reticulo-endothelial elements. Warthin,<sup>10</sup> MacCarty<sup>11</sup> and others are of a similar opinion. Krumbhaar's<sup>42</sup> recent report suggests an origin in the reticulo-endothelial system. It seems to rule out the origin strictly in lymph nodes and to point to the more universal reticulo-endothelial-like cells as the origin. It is difficult on this basis, however,

41. Maximow, A.: *A Text-Book of Histology*, Philadelphia, W. B. Saunders Company, 1930, p. 104.

42. Krumbhaar, E. B.: *Am. J. M. Sc.* **182**:764, 1931.



to reconcile Warthin's observations on the transformation of Hodgkin's disease to lymphosarcoma. There is no reason, however, why the giant cells may not arise as totipotent "lymphoblast-like" or "reticulo-endothelial-like cells" and become altered as in Hodgkin's disease to resemble megakaryocytes. In fact, Bloom<sup>43</sup> stated that Maximow grew myeloid cells in cultures of lymph node cells. Nevertheless, this does not prevent bone marrow cells from being filtered out as in any other infection, but true megakaryocytes may not always be present. This is perhaps similar to the state of affairs existing with the eosinophils. At present it seems better to wait for more evidence before drawing final conclusions.

In the metastases to the liver in the case reported there was practically one type of cell that could be followed from a lymphoid-like cell to large giant cells. More complete details of this, however, must be reserved for a separate study.

#### SUMMARY AND CONCLUSION

An extremely early primary pulmonary tuberculous complex occurring near the end of a case of advanced Hodgkin's disease has been described. Two characteristic types of lesions were found in the same body: a small early tuberculous focus in the lungs and adjacent lymph nodes, and advanced Hodgkin's disease in the cervical and peripancreatic lymph nodes, liver and spleen. The lesions in the tissue showing Hodgkin's disease resembled malignant changes of lymphoid cells similar to those originating in the lymph nodes. Only human tubercle bacilli were isolated from the lesions of Hodgkin's disease. No filtrable forms could be demonstrated.

It seems justifiable to conclude that Hodgkin's disease in the patient studied developed independent of tuberculous infection, and that the theory of a tuberculous origin of the disease is not supported by this case.

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43. Bloom, William: *Proc. Inst. Med.*, Chicago 8:322, 1931.

# EXPERIMENTAL PATHOLOGY OF THE LIVER

## VI. RESTORATION OF THE LIVER IN WHITE RATS AFTER PARTIAL REMOVAL AND SPLENECTOMY<sup>\*</sup>

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The fixed histiocytes of the animal organism are, to a large extent, localized in the liver, spleen, lymph nodes and bone marrow. Following ablation, either surgical or pathologic, of any portion composed of these cells, compensatory hyperplasia of the remaining component parts usually ensues. In a measure, the Kupffer cells of the liver and the reticular cells of the red pulp and splenic sinuses are complementary. Lepehne<sup>1</sup> stated that the relatively small spleen of birds has its counterpart in the extensive Kupffer cell system of the liver, and that splenectomy in mammals induces in the liver an avian type of histiocytes. Studies of the liver following removal of the spleen,<sup>2</sup> as well as after ligation of both splenic blood vessels,<sup>3</sup> indicate rather clearly that the hepatic changes are largely compensatory. Such splenic functions as metabolism of iron pigment, pursuant to destruction of blood, and metabolism of lipoids are taken over by the increased number and the hyperplasia of Kupffer cells in the liver.<sup>4</sup>

Since the liver had been shown to increase in volume<sup>5</sup> following removal of the spleen, probably coincidently with extensive metaplasia of the histiocytes, we conducted experiments to learn whether the rate or the extent of the restoration of the liver following partial removal would be modified in any way by coincident removal of the spleen.

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<sup>\*</sup> Submitted for publication, Nov. 4, 1931.

<sup>\*</sup> From the Division of Experimental Surgery and Pathology, the Mayo Foundation.

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3. Romanenko, Peter: Ueber pathologisch-histologische Veränderungen an den inneren Organen des Hundes nach der Unterbindung der Milzgefasse, Arch. f. klin. Chir. **153**:123, 1928.

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Throughout this paper, frequent references will be made to a previous report (Higgins and Anderson<sup>6</sup>). In large part, these references will be for the purpose of allowing comparison between the results of the present work, in which operation involved both liver and spleen, and the former work in which only the liver was concerned.

#### EXPERIMENTAL METHOD

It has previously been shown<sup>6</sup> that, following surgical removal of 75 per cent of the rat's liver, restoration begins, as evidenced by hypertrophy and mitosis, the latter part of the first day, and following a cyclic activity, the normal ratio of body weight to liver weight is restored within from ten days to two weeks. The technic of partial removal has been described.

In the present investigations, a series of eighty rats was operated on. Only healthy white rats, aged from 6 to 9 months, and ranging in weight from 125 to 225 Gm., were used.

On the basis of the formula, hitherto determined,  $y = 0.024x + 2.1 \pm \frac{0.5752}{n-1}$ , in which  $y$  is liver weight and  $x$  is body weight, the average weight of the liver in the series was  $5.786 \pm 0.64$  Gm. The mean weight of liver resected was  $4.06 \pm 0.0620$  Gm., which was approximately 70 per cent of the total hepatic parenchyma. Thus, an average remnant of liver weighing  $1.726 \pm 0.0890$  Gm. remained in the body.

For some reason as yet unexplained, splenectomy with partial hepatectomy raises the mortality. In our experience with partial hepatectomy in rats, a mortality of from 20 to 25 per cent is explained by postoperative pulmonary disturbances rather than by loss of the liver. If the spleen is removed coincidentally with the portion of the liver, as many as from 40 to 50 per cent of the animals may succumb in the ensuing forty-eight hours.

Animals that survived operation, forty in number, were maintained on the standard laboratory ration, and groups of them were killed by exsanguination at seventy-two hours, seven days, fourteen days, twenty-one days and twenty-eight days. The weights of the animals and the weights of the moist livers were recorded, and portions of the hepatic parenchyma were fixed and stained with hematoxylin and eosin, eosin azur II and Mallory's stain for connective tissue. The data on the weights of the animals and the weights of the livers which were assembled during the period of observation have been condensed into the accompanying tabulation.

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6. Higgins, G. M., and Anderson, R. M.: Experimental Pathology of the Liver: I. Restoration of the Liver of the White Rat Following Partial Surgical Removal, *Arch. Path.* **12**:186, 1931.

## EXPERIMENTAL OBSERVATIONS

*Weight of Liver.*—A transient fall in the weight of the body occurred following operation. This had no particular significance, since it occurred when simple laparotomy, as well as when simple hepatectomy was performed. The maximal loss was attained at about one week after operation, and complete recovery, ordinarily with some gain in weight, was experienced between the second and the third week. The animals continued healthy, so that in the latter periods of observation,

*Mean Weights of Body and of Moist Liver Before Splenectomy and Partial Hepatectomy and at Intervals During Restoration*

Group	Animals	Lapse of Time After Operation Before Animals Were Killed, Days	Mean Weights							
			Before Operation		At Time of Splenectomy and Partial Hepatectomy		At Time of Death		During Restoration	
			Body, Gm.	Liver, Gm.	Liver Removed, Gm.	Liver Remaining, Gm.	Body, Gm.	Liver, Gm.	Actual Increase of Liver, Gm.	Weight of Liver, per Cent of Body Weight
All operated on	80*	..	153.6 ± 2.25	5.786 ± 0.064	4.06 ± 0.0620	1.726 ± 0.0890				
1	5	3	151.8 ± 8.96	5.743 ± 0.2876	3.98 ± 0.1123	1.76 ± 0.3087	147.6 ± 9.020	4.46 ± 0.1956	2.70 ± 0.3654	0.0302
2	10	7	150.4 ± 2.21	5.709 ± 0.2876	4.48 ± 0.1112	1.229 ± 0.3083	128.8 ± 7.160	5.40 ± 0.2327	4.26 ± 0.3862	0.0426
3	10	14	158.4 ± 7.50	5.901 ± 0.2876	4.24 ± 0.0825	1.661 ± 0.2992	149.4 ± 5.580	6.13 ± 0.3438	4.47 ± 0.4557	0.0410
4	7	21	160.6 ± 13.70	5.954 ± 0.2876	4.30 ± 0.3102	1.654 ± 0.4230	182.8 ± 13.190	7.32 ± 0.4987	5.66 ± 0.6538	0.0400
5	8	28	152.2 ± 8.55	5.752 ± 0.2876	3.72 ± 0.2524	2.032 ± 0.3820	172.6 ± 8.22	6.41 ± 0.2785	4.37 ± 0.4727	0.0371

\* Forty survived operation.

weight of body was recorded at from 12 to 20 per cent above the pre-operative level.

When the weights of the liver at the intervals noted were plotted, and the ratios of the weight of the liver to the weight of the body were recorded and comparisons made with the curve of restoration of the liver following partial hepatectomy only,<sup>6</sup> a contrast was at once apparent. On the basis of weights and ratios, it was clear that hepatic tissue was restored in somewhat larger amount and more rapidly if the spleen had been removed. In computations of the weight of the body, loss of the spleen was ignored. It seldom weighed more than 0.8 Gm., and its weight would have no appreciable effect on the ratio of the weight of the liver to the weight of the body.

In the first seventy-two hours after the operation, the mean increase in hepatic parenchyma was  $2.70 \pm 0.3654$  Gm., or an increase of approximately 1.8 Gm. for each 100 Gm. of the weight of the body. Although computations of the extent of restoration were not made at the twenty-four hour and the forty-eight hour interval, it is interesting to note that the amount of parenchyma restored at seventy-two hours, following partial removal and splenectomy, was essentially that attained at the same interval following partial hepatectomy alone, as shown in our earlier report.<sup>6</sup> In the splenectomized rats, however, the mean body weight of those killed after seventy-two hours was 12 Gm. less than

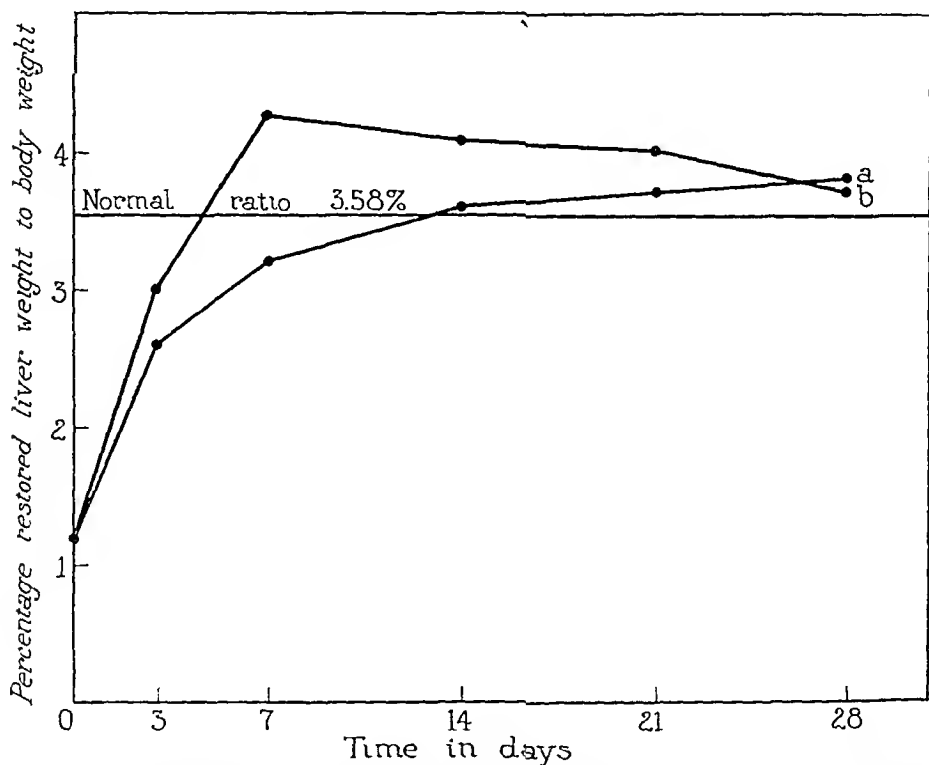


Fig. 1.—Percentages of weight of liver in relation to weight of body during restoration.

the mean body weight of the hepatectomized rats, killed at the same time; so that the ratio of liver weight to body weight in the splenectomized series was 0.3 per cent higher at the end of the third day, as shown in figure 1.

At the end of a week, a more significant difference had occurred. When partial hepatectomy alone was done, the weight of the livers of the rats killed after seven days was  $4.51 \pm 0.1227$  Gm. which was an actual increase, during that interval, of  $2.69 \pm 0.2440$  Gm., as shown in the tabulation of our original study.<sup>6</sup> In contrast to this, splenectomized animals, after seven days of restoration, had hepatic parenchyma

weighing  $5.49 \pm 0.2327$  Gm., which was an actual increase of  $4.26 \pm 0.3862$  Gm. during the week. This increment represents an increase of 2.8 Gm. for each 100 Gm. of preoperative body weight; or, in contrast to the series of simply hepatectomized animals, it appeared that when splenectomy accompanies partial removal of the liver, parenchyma is restored, at seven days, equal to 1.2 Gm. for each 100 Gm. of body weight, more than when the spleen is left intact. The loss of body weight was more or less constant for the two groups, and the ratio of liver weight to body weight in the splenectomized rats, at the end of a week, was 1.1 per cent higher than that in rats with the spleen intact.

In contrasting the data on the weight of the liver in the two groups after fourteen days of restoration, the difference in the actual increase of liver or in the ratio of liver weight to body weight is far less significant. When the liver only was removed,<sup>6</sup> the actual increase in restored parenchyma at fourteen days was 2.3 Gm. for each 100 Gm. of preoperative body weight; if splenectomy also was done, the increase in parenchyma for each 100 Gm. of body weight was 2.8 Gm., which represents a restoration in the splenectomized rats greater by only 0.5 Gm. for each 100 Gm. of body weight. Accordingly, the ratios of liver weight to body weight of the two more nearly coincide at the fourteenth than at the seventh day, diverging from each other at the fourteenth day by only 0.6 per cent.

Again, twenty-one days after operation, the data indicate that a greater increase of parenchyma had occurred following splenectomy and partial hepatectomy than following simple hepatectomy. On the basis of preoperative weight of the body, the restoration in the latter group was 2.6 Gm. for each 100 Gm. of body weight,<sup>6</sup> whereas an increase of 3.5 Gm. for a corresponding unit was observed in rats without spleens. Since the increase in body weight over the preoperative value was greater at twenty-one days in the splenectomized animals than in those from which the liver only was removed, the ratios of liver weight to body weight were less divergent than the disparity in the weights of the restored parenchyma would lead one to conclude. Accordingly, the ratio of weight of liver to weight of body was 0.1 per cent lower than at fourteen days, and only 0.5 per cent higher than that of the group from which the liver component only was removed.

Data assembled at the end of the twenty-eighth day, based on eight rats which had survived the entire period of restoration, were somewhat changed from those compiled after three weeks. In the ratio of weight of liver to weight of body there was a fall of 0.29 per cent from that recorded after twenty-one days of observation; so that the ratio was slightly below that attained after twenty-eight days in the animals from

which only the liver component had been removed.<sup>6</sup> These animals continued in good health, and an increase of 13 per cent in weight of body over the preoperative weight of the body was attained. The reasons for the decrease in the amount of the hepatic parenchyma at the twenty-eighth day are not clear.

*Cytology.*—One of the earliest responses in the liver to removal of the spleen is marked hyperemia. In earlier studies of changes induced by splenectomy, an increase in the size of, and a severe congestion of, the liver was noted within twenty-four hours following operation. Sub-

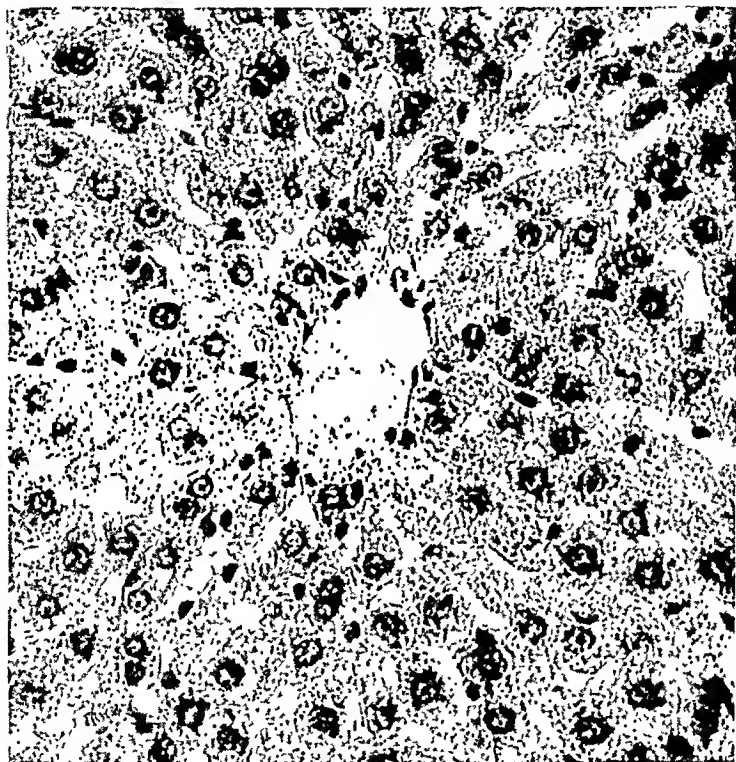


Fig. 2.—Lobule of liver of adult white rat, showing normal distribution of histiocytes;  $\times$  425.

sequent changes included lymphocytic infiltration and development of lymphoid foci with splenic potencies within the liver. Marked hyperplasia of the histiocytes invariably followed splenectomy—evidence of the assumption by the liver of certain well known splenic functions.

In this study of restoration of the hepatic remnant following splenectomy, we wished to know whether the coincident removal of the hepatic component would induce within the hepatic remnant any additional cytologic changes that were not encountered in this organ when only the spleen was removed. Accordingly, thirty rats were operated on. Ten of these were subjected to simple partial hepatectomy, ten to

removal of the spleen and ten to removal of both the spleen and the hepatic component at the same time. The animals were killed at intervals corresponding to those selected for the accumulation of data on restored liver weight, ranging from twenty-four hours to four weeks.

At twenty-four hours after removal of both the spleen and the hepatic component, histiocytic compensation in the hepatic remnant was only slightly evident. The number of Kupffer cells in the sinusoids had definitely increased over that in the hepatic remnant of the animals with intact spleens. The necessity for so early a histiocytic hypertrophy was not clear, for there was no evidence that metabolism of pigment had

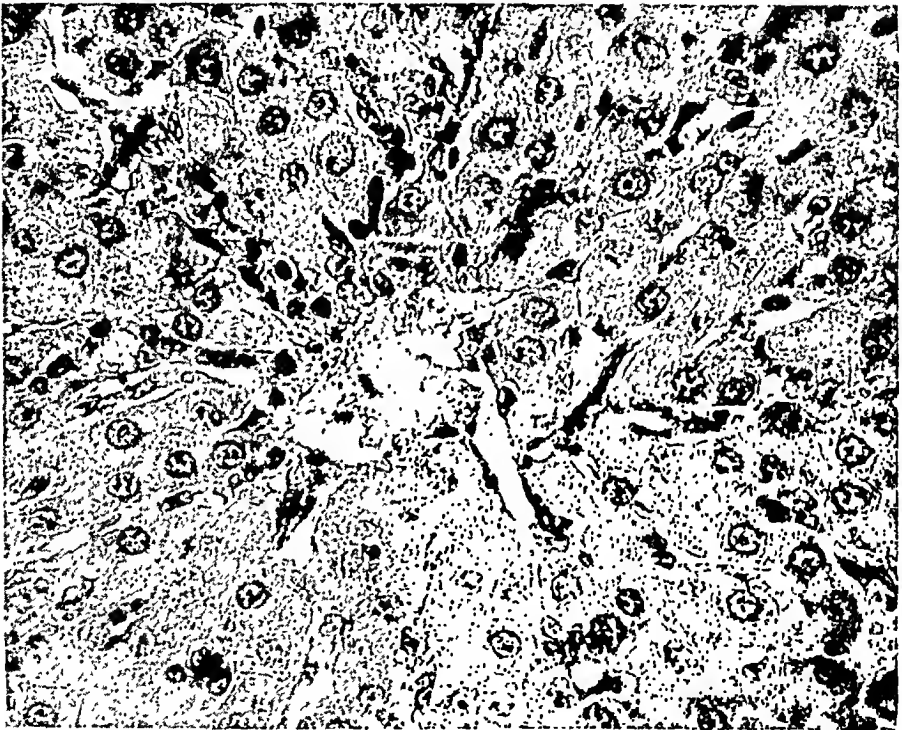


Fig. 3.—Lobule of liver of white rat ninety-six hours after removal of spleen and partial hepatectomy; increase in number of histiocytes is shown;  $\times 425$ .

been increased. The method of increase in the number of these histiocytes was not definitely determined, although amitosis was strongly indicated. There were considerable vacuolation and cloudy swelling of hepatic cells in the livers of all animals killed in the early postoperative period, and occasional eosinophilic leukocytes were identified in the sinusoids of the splenectomized animals.

At forty-eight hours following operation, generalized inflammation characterized the hepatic remnant in all the animals. More marked injury occurred in the livers of the animals from which only the spleens had been removed. The cells were vacuolated, and the cytoplasmic



bodies were heavily granulated, a condition that always accompanied splenectomy. The sinusoids were hyperemic. In the hepatic remnant of the animal from which both spleen and liver had been removed, cytologic injury was less marked. This, we feel, may be due to factors coincident with restoration of the hepatic parenchyma. Cellular nuclei of the hepatic remnant were hypertrophic; early prophase were numerous, and occasional mitotic figures were seen. It may be that the metabolic activity indicated by mitosis protected these hepatic cells against the degree of injury that splenectomy imposed on the intact liver. Kupffer cells were far more active in the hepatic remnant of the animal that was without both spleen and hepatic component, and at forty-eight hours they were far more abundant than at the twenty-four hour interval.

The most marked cellular reaction in these thirty livers occurred on the fourth day following operation. Hepatic parenchyma increased mitotically, and binucleate cells were common, but proliferation of the hepatic cell was no more marked in animals which had undergone both splenectomy and hepatectomy than in animals from which the liver only had been removed. The total weights of restored liver at this period, however, were greater in the series of animals that had undergone splenectomy. Cytologically, then, the increase in the weight of the liver in the splenectomized animals was essentially due to the new histiocytic elements developing in the liver as compensation for the loss of the spleen. The histiocytes (figs. 2 and 3) were triple their normal size, and many of them contained numbers of engulfed red blood cells. These cells were actively proliferating, and several mitotic figures of dividing Kupffer cells were identified. Furthermore, as a compensatory reaction, isolated foci of lymphoid tissue, so abundant in later stages, first appeared at this time. Nests or groups of small lymphocytes had accumulated extravascularly along the hepatic trabeculae in various portions of the lobule; or they were often encountered directly in the sinusoid, attached to one or more histiocytes (figs. 4 and 5). Lymphoid tissue was not identified in the intact liver of the splenectomized animal as early as in the restored liver following partial removal. This may be due to the reduced parenchyma available for such compensation in partially hepatectomized animals. One would be more likely to encounter lymphoid tissue in a reduced hepatic parenchyma, where the concentration of lymphoid tissue is likely to be the greater. Likewise, the histiocytic compensation appeared to be far more extensive in the restored livers following partial removal than in the intact livers of splenectomized animals. In the former, the extent of the phagocytosis of whole or of fragmented red cells is enormous, owing, we feel to an excessive load placed on these cells, which constitute, following opera-

tion, practically one fourth of the number in a normal liver. Metabolism of pigment in the liver was greatly increased in these hepatectomized and splenectomized animals. Eosinophilic polymorphonuclear leukocytes were fairly abundant in both series of splenectomized animals, but they were only rarely seen in animals with intact spleens. They occurred around the portal vessels, especially in the restoring livers, and constituted a goodly percentage of the mesenchymal infiltration.

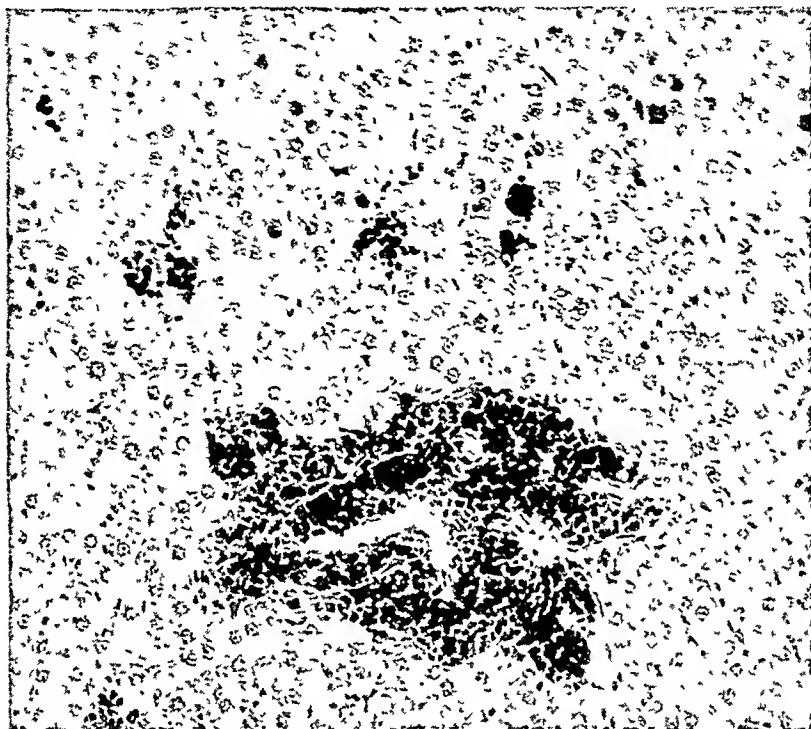


Fig. 4.—Liver of white rat four weeks after removal of spleen and partial hepatectomy; cellular infiltration around portal spaces and foci along sinusoids are shown;  $\times 240$ .

Cytologic study of the liver that was the product of restoration, at two, three and four weeks, showed only further development of conditions inaugurated in the first few days. A normal hepatic parenchyma was not completely restored, for slight granulation and vacuolization of the cytoplasm still persisted at four weeks. Portal spaces were not normal, for many lymphocytes and polymorphonuclear leukocytes surrounded bile ducts and venous radicles. Reticular cells were abundant, and numbers of foci of developing myeloid components abounded both within the sinusoids and along the trabeculae in the liver four weeks after partial removal.



Fig. 5.—Liver of white rat four weeks after removal of spleen and hepatic component; hemopoietic foci are shown along sinusoid;  $\times 1220$ ; Bausch and Lomb 3 mm., ocular  $\times 10$ .

## COMMENT AND SUMMARY

Since the liver and the spleen are in a measure complementary in that certain splenic functions are often assumed by comparable cells in the liver, a study has been made of the restoration of the liver following partial surgical removal and splenectomy. Eighty rats were subjected to operation by which about 70 per cent of the hepatic parenchyma and the entire spleen were removed, resulting in a mortality of 50 per cent. Those rats which survived the operation were killed at definite intervals, as shown in the tabulation, ranging from three days to four weeks, forming the experimental series from which the data have been derived. In a previous paper<sup>6</sup> results of partial removal of the liver only have been reported, and may be used in comparison with the results of the present study.

Restoration of the hepatic remnant began on the first day, and at the end of the third day the ratio of weight of liver to weight of body was slightly higher among the animals from which the spleen and the liver component had been removed than among those which had undergone partial removal of the liver only.<sup>6</sup> At one week following operation, the extent of restoration of the hepatic remnant was vastly greater in the experimental series in which partial removal of the liver was accompanied by splenectomy. In this series, a parenchyma greater by 1.2 Gm. for each 100 Gm. of body weight was restored. This difference was, to a certain extent at least, one of cytology; as a compensation for the loss of the spleen a marked increase in the number and size of the histiocytes had occurred.

From the high ratio of the weight of the liver to the weight of the body (0.0426) encountered at the end of the first week, when the two curves of restoration were more widely divergent than at other times, there was a gradual decline, until at twenty-eight days the ratios were essentially the same. The distribution of histiocytes was not so great during the latter periods of restoration, but the nests of lymphocytic foci, such as are shown in figure 5, were frequent. It is clear that these hepatic remnants, during restoration after splenectomy, became hemopoietic centers, but the data do not indicate that this cytologic compensation is sufficient to account for the disparity in the curves of restoration.

# THE ACTION OF ANTILEUKOCYTIC SERUM ON TISSUE CULTURES \*

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In spite of the fact that the tissue culture method, which has been known for about twenty years, has opened a new field for the study of the various immunologic aspects of cytotoxins, relatively little use has so far been made of this method of investigation.

The first cytotoxic studies on tissue cultures were made by Lambert and Hanes<sup>1</sup> in 1911, who experimented with cytotoxic serum against malignant tumor cells of rats and mice. Work on similar lines has been done in recent years by Lumsden.<sup>2</sup> Investigations on the effects of an antifibroblastic serum, obtained by the injection of chick embryo pulp into rabbits, have been carried out by Kimura<sup>3</sup> and Fischer.<sup>4</sup>

We wish to report a series of observations made in various tissue explants cultured in antileukocytic plasma or serum produced in rabbits by repeated intravenous injections of human, chicken and beef leukocytes.

## PREPARATION OF ANTILEUKOCYTIC SERUMS

For the production of antileukocytic serum for other purposes different sources of leukocytic supply have been used by previous investigators (bone marrow by Besredka,<sup>5</sup> Flexner<sup>6</sup> and Bunting;<sup>6</sup> spleen and lymph node by Metschnikoff,<sup>7</sup> Funk<sup>6</sup> and Flexner;<sup>6</sup> aseptic purulent exudate from the intraperitoneal injection of aleuronat by Gladin<sup>8</sup> and Lindstroem;<sup>9</sup> that from the intraperitoneal injection of

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\* From the Cancer Research Laboratories, University of Pennsylvania Graduate School of Medicine.

1. Lambert and Hanes: J. Exper. Med. **14**:453, 1911.

2. Lumsden: Lancet **1**:383, 1924; **1**:112, 1926; Arch. f. exper. Zellforsch. **6**:206, 1928; Am. J. Cancer **15**:563, 1931; J. Path. & Bact. **34**:349, 1931.

3. Kimura: Arch. f. exper. Zellforsch. **6**:185, 1927; Ztschr. f. Immunitätsforsch. u. exper. Therap. **55**:501, 1928.

4. Fischer: Gewebzüchtung, ed. 3, Munich, Rudolph Müller & Steinicke, 1931.

5. Besredka: Local Immunity, Baltimore, Williams & Wilkins Company, 1927.

6. Quoted by Fischer (footnote 4).

7. Metschnikoff: Ann. Inst. Pasteur **14**:369, 1900.

8. Gladin, quoted by Lindstroem (footnote 9).

9. Lindstroem: Acta med. Scandinav., supp. 22, 1927.

beef bouillon by Spaet and Holder<sup>10</sup> and Yamamoto;<sup>11</sup> that from the intraperitoneal injection of staphylococcus vaccine by Borgi;<sup>12</sup> that from the subcutaneous injection of turpentine oil by Spanier;<sup>13</sup> that from the intraperitoneal injection of pilocarpine, which gave a predominantly monocytic exudate, and that from the intraperitoneal injection of aqueous extract of *Ascaris lumbricoides*, which gave an eosinophilic exudate, by Yamamoto;<sup>11</sup> and the buffy coat of centrifugated noncoagulated blood by Carrel and Ebeling,<sup>14</sup> Lindstroem<sup>9</sup> and ourselves.<sup>15</sup> As a matter of convenience and specificity, we chose the crusta phlogistica of centrifugated, nonclotted blood to furnish the leukocytes for the injection into the rabbits.

From 10 to 15 cc. of leukemic blood or 40 cc. of normal blood supplies the necessary amount of leukocytes for one or two injections. The coagulation of the blood is prevented by the addition of heparin or sodium citrate. After the blood has been centrifugated and the supernatant plasma has been discarded, the gray buffy coat covering the erythrocytic sediment is either carefully removed with a pipet or, if clotting has occurred or has been induced by the addition of "calcium" Ringer's solution,<sup>16</sup> with a forceps. The leukocytes are then thoroughly and repeatedly washed in Ringer's solution to remove as much as possible the adherent erythrocytes. This procedure is an important step in the preparation of antileukocytic serum, as the presence of erythrocytes in the material to be injected results in the production of a serum with hemolytic qualities. It is for this reason that antileukocytic serums obtained by the injection of bone marrow or splenic tissue have also undesired hemolytic effects.

After the washing, the leukocytes are suspended in about 10 cc. of Ringer's solution. The suspension usually has a light milky appearance and is slightly pink. If standardized amounts of leukocytes are to be injected, the number of cells present in the suspension can be counted. In case the clotted leukocytes are to be used, the buffy coat is ground in a mortar, with a small amount of Ringer's solution added. After a soft, loose pulp has been obtained, the material is squeezed through several layers of sterile gauze, to remove the coarser particles. The filtrate is suspended in 10 cc. of Ringer's solution. The material is then ready for intravenous injection. The injections are given twice a week. A weakly antileukocytic serum is usually obtained with five injections. But from 10 to 15 injections are in general needed for the production of a strongly antileukocytic serum.

10. Spaet and Holder: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **49**:383, 1926. Spaet: *ibid.* **49**:382, 1927.

11. Yamamoto: *Tohoku J. Exper. Med.* **15**:324, 1930.

12. Borgi: *Krankheitsforschung* **8**:308, 1930.

13. Spanier: *Beitr. z. Klin. d. Tuberk.* **73**:210, 1930.

14. Carrel and Ebeling: *J. Exper. Med.* **36**:365, 1922.

15. Hueper and Russell: *Arch. Int. Med.* **49**:113 (Jan.) 1932.

16. Ringer's solution, containing calcium chloride, is made as follows:

2.0 per cent sodium chloride solution (NaCl).....	475 cc.
0.2 per cent potassium chloride solution (KCl).....	100 cc.
4.0 per cent calcium chloride solution (CaCl <sub>2</sub> ).....	90 cc.
Water .....	335 cc.

1,000 cc.

The testing and titration of the serum of the animals thus prepared for antileukocytic qualities can be properly done only by the tissue culture method, as the cytotoxic qualities of a given serum cannot be correctly evaluated by agglutination, precipitation or complement-fixation (Lindstroem,<sup>9</sup> Lambert,<sup>1</sup> Foot<sup>17</sup>).

The clotted crusta phlogistica of the blood of the leukocytic donor is cut into small pieces, which are explanted in the antileukocytic plasma or serum, respectively. The plasma is used full strength and in various dilutions with plasma from a normal animal or with Ringer's or Tyrode's solution. From ten to twelve cultures are used for the testing of each dilution. The presence of antileukocytic qualities in the plasma to be tested is evidenced by a complete or partial inhibition of the emigration of cells from the explant. The antileukocytic titer of the serum is represented by the highest dilution of the plasma or serum that still inhibits completely the emigration of leukocytes from at least 75 per cent of the explants of the respective set.

The titration of the plasma is important, because of the marked variations in the antileukocytic qualities of the serum of different animals identically prepared. It is also advisable to repeat the titration, if the animal has been bled repeatedly and at short intervals, as the titer has then a tendency to drop. This precaution is essential for comparative results. Parenteral injections of protein result, on the other hand, in a temporary rise of the antileukocytic qualities of the serum.

Anaphylactic reactions were observed only twice after the injection of leukocytes intravenously. They were not fatal, and did not occur if the injections of the cellular suspensions were slowly performed. The sterile handling of the leukocytic material used for injection is important, as otherwise local infections of the ear and bronchopneumonia may result from the injection of contaminated leukocytic suspensions.

The following five antileukocytic serums were prepared according to the method outlined and were used in the experiments to be described:

1. Human toxic antileukocytic serum produced by the injection of normal human leukocytes into rabbits.
2. Human toxic antileukemic serum produced by the injection of leukocytes from a patient with chronic myeloid leukemia into rabbits.
3. Chicken toxic antileukocytic serum produced by the injection of normal chicken leukocytes into rabbits.
4. Chicken toxic antileukemic serum produced by the injection of leukocytes from chickens with myeloid leukemia into rabbits.
5. Beef toxic antileukocytic serum produced by the injection of beef leukocytes into rabbits.

The leukemic chickens used were Barred Rocks affected by the transmissible type of myeloid leukemia. The disease had been transmitted to them by the intravenous injection of 2 cc. of blood from a leukemic chicken, which was

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17. Foot: *Centralbl. f. allg. Path. u. path. Anat.* **23**:578, 1912.

obtained through the courtesy of Dr. E. L. Stubbs of the Veterinary School of the University of Pennsylvania. About four weeks after the inoculation of the leukemic blood, the combs of the chickens lost their turgor, became drooping and changed in color from the normal bright red to a distinct reddish yellow. The animals became weak and lost their appetite. Examinations of the blood made at this time showed a marked increase in the number of leukocytes and the appearance of immature forms. The chickens were then bled to death from their carotid arteries. The leukocytes thus obtained were used for explantation and injection. Subsequent histologic examination of the spleen, liver and bone marrow showed in three of ten chickens given injections a marked leukemic infiltration of these organs. In one of these animals, a diffuse and nodular leukemic involvement of the stomach was seen, and in another animal a similar condition was found in the heart muscle. In the organs of the remaining seven animals, no definite evidence of leukemia could be discovered.

#### OBSERVATIONS

When normal or leukemic human or chicken leukocytes were explanted in human toxic or chicken toxic antileukocytic or antileukemic plasma, respectively, the following observations were made:

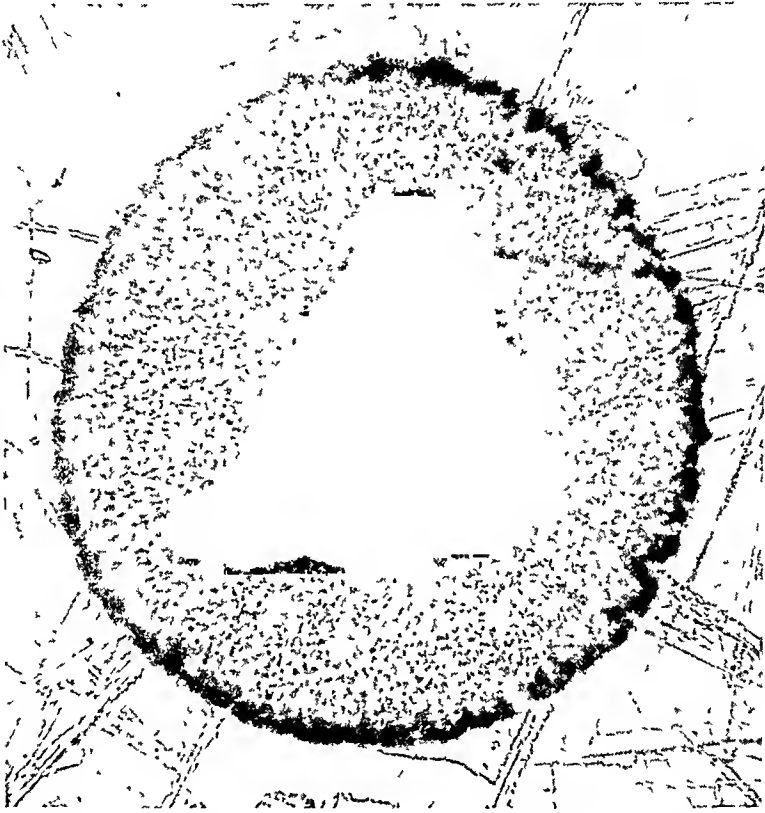
A potent antileukocytic or antileukemic plasma, respectively, inhibited completely the emigration and proliferation of leukocytes from the explants. These showed sharp outlines and had after forty-eight hours' incubation sometimes a homogeneous, coagulated-like appearance. After prolonged incubation, a gradual disintegration of the explanted leukocytic clot occurred. The plasma in the immediate vicinity of the explant was somewhat less opaque than that in the peripheral parts of the plasma clot. In this region the plasma contained a dense network of fibrin and, embedded in it, very delicate, reflecting granules. In cultures set up in an antileukocytic plasma of lower potency or in dilutions of a potent plasma, emigration and proliferation of cells were observed. The diameter of the zone of emigration and its density depended, however, on the antileukocytic strength of the plasma. In a weakly antileukocytic plasma, the zone of emigration was wide and the cells were loosely scattered in it, while in a stronger antileukocytic plasma the zone was narrow and the emigrated cells were densely packed in it. The cells were usually conglomerated in small clumps. The most striking picture was, however, offered by the peripheral portions of the emigration zone. It appeared in the living culture, on macroscopic examination, as a sharply defined, chalky, white ring and consisted of conglomerated leukocytes. Nuclear fragments, cellular detritus and leukocytes without nuclei were numerous and scattered throughout the zone of emigration. While an emigration of cells beyond the peripheral ring was usually absent, sometimes large, swollen cells without nuclei were found there (cell shadows).

After from three to four days of incubation, progressive decomposition began in the cells of the emigration zone, changing it into an area of cellular débris. This process could be precipitated, if after twenty-



four hours' incubation a drop of antileukocytic serum was added to the culture. In the course of a few hours, signs of degeneration of the emigrated cells became visible, which ultimately resulted in a complete destruction of the leukocytes in the area of emigration. After a primary shrinkage of the cells, they broke up into finely granular material. An immediate and direct cytolysis was not observed in any instance.

It was furthermore demonstrated through cross-cultures of normal leukocytes in antileukemic plasma and leukemic leukocytes in antileuko-



Leukocytic culture in a medium of 25 per cent antileukocytic plasma plus 75 per cent normal plasma, showing the restricted emigration of the leukocytes and the dense peripheral ring of conglomerated cells.

cytic plasma that antileukemic plasma and leukemic leukocytes do not possess any leukemic specificity. The antileukemic plasma was not less cytotoxic against normal leukocytes than normal antileukocytic plasma was found to be. The same relation existed in regard to the efficacy of normal antileukocytic serum against leukemic leukocytes, as long as the leukocytes were tested against an immune serum prepared with leukocytes of the same species. In view of the fact, however, that the antileukemic serums used were produced by the injection of predominantly myeloid cells, it must remain at present an open question whether or

not serum of this type will be active against lymphocytes and monocytes. This appears to be rather doubtful on account of the experiments of Yamamoto<sup>11</sup> on leukocytic type-specific antisera.

As Fischer<sup>4</sup> had asserted that cytotoxic serum possesses only an antiproliferative, and not a cytolethal, effect on cells in tissue cultures, a reexamination of this question seemed to be indicated. Fischer<sup>4</sup> based his statement on the observation that fibroblast that had been exposed through three passages to an antifibroblastic serum started to proliferate when they were brought into normal plasma. This claim was made by Fisher to contradict a previous statement of his pupil Kimura<sup>3</sup> concerning the cytolethal action of antifibroblastic serum. Our observations, as well as those of Foot<sup>16</sup> on bone marrow cultures, definitely prove, however, that cytotoxic immune serum has a cytolethal effect. When leukocytic cultures that had been grown first in normal plasma were transplanted into antileukocytic plasma, a rapid and definite degeneration and necrosis of the cells in the zone of proliferation was observed. The cells shrank, broke up into small fragments and finally disappeared, leaving only a zone of cellular débris around the explant. This zone contained fine, densely packed granules. When, on the other hand, leukocytic cultures that had been exposed to antileukocytic plasma and that had not shown any evidence of cellular emigration or proliferation were transplanted into normal plasma, no emigration occurred during the first twenty-four hours of incubation and only a scanty one in a small percentage of the cultures after forty-eight hours of incubation. It was, moreover, noticed that the cells which then emigrated were pathologic in many respects and seemed to emigrate from the central portions of the explants. The cells were about three times as large as those normally seen at this time, contained large, densely packed droplets and were rather closely attached to the explant. In subsequent experiments with fibroblastic cultures from the heart, muscle, cartilage and spleen of the same species (chicken), identical observations were made. The delayed and scanty growth of cells following exposure of the explant to an immune serum in a certain percentage of the cultures can be satisfactorily explained by the fact that the cytotoxic serum had not killed the cells in the central portions of the explant. The potency of the immune serum is certainly another important factor in regard to the cytolethal efficacy of the serum used.

But there exists also sufficient clinical evidence of the cytolethal effect of antileukocytic serum according to the investigations of Lindstroem<sup>9</sup> and Yamamoto.<sup>11</sup> Both observed, after injection of antileukocytic serum into animals, a marked leukopenia resembling that seen in agranulocytosis. Lindstroem noted, moreover, an absence of leukocytes in the bone marrow of animals thus treated and killed by the effect of the injection received. Lindstroem utilized his observations on

animals in the therapeutic application of antileukocytic serum in leukemia. He obtained prolonged remissions in four of eleven cases in which this treatment was employed. He pointed out that his failure to get better and more uniform results was partly due to the fact that a proper titration of the serum used was not possible, so that a weakly antileukocytic serum was sometimes injected, followed by aggravating results, because the leukocytotoxic effect was overcompensated by the normal stimulating hematopoietic effect of the serum injected.

The species-specific qualities of the antileukocytic rabbit serums used were readily demonstrated, when tissues of various chicken organs (spleen, heart, cartilage, skin, intestine) were cultured in the plasmas prepared against chicken, human and beef leukocytes (Witebsky and Kosmiya <sup>18</sup>). While there was a complete inhibition of cellular emigration and proliferation in the cultures embedded in the plasma prepared against chicken leukocytes, the explants in that prepared against human and beef leukocytes grew as well as those seeded in normal plasma.

A selective or more pronounced inhibitory or toxic action of antileukocytic serums against mesenchymatous than against epithelial cells was not noticed, when skin and intestine were explanted in different dilutions of species-specific antiserum and in nonspecies-specific antiserum. Antileukocytic serum can therefore not be used as an agent that might facilitate the culturing of pure strains of epithelial cells.

Antileukocytic serums possess, however, apparently to a moderate degree, nonspecies-specific-organ-specific qualities. It was repeatedly observed that leukocytes explanted in a nonspecies-specific antiserum showed a definite, though slight to moderate, inhibition of emigration. This action seemed to depend on the potency of the antileukocytic serum used. As an illustration of this observation, the following planimetric determinations of the zones of emigration of leukemic chicken leukocytes in normal rabbit plasma in that prepared against human leukocytes and in normal chicken plasma may be cited.

Medium	Growth Coefficient
Normal chicken plasma.....	8.78
Normal rabbit plasma.....	4.10
Rabbit plasma prepared against human leukocytes.....	1.70

Any disease-specific action of antileukemic serum on the leukemic leukocytes of another species was not observed. Serum prepared against the leukocytes of leukemic chickens will therefore be ineffective, in all probability, in human leukemia.

Finally the leukocytes of the rabbits, the serum of which had been rendered antileukocytic, were tested for antileukocytic constituents. The antileukocytic rabbits were bled to death and the crusta phlogistica

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18. Witebsky and Kosmiya: Ztschr. f. Immunitätsforsch. u. exper. Therap. 67:480. 1930.

obtained from their blood was ground in a mortar with a few cubic centimeters of Tyrode's solution. After filtration of the soft leukocytic pulp through filter paper, the filtrate was diluted to about 5 cc. with Tyrode's solution. Equal parts of the filtrate were added to normal plasma and used as a test medium in leukocytic cultures. It was found that the leukocytic extract of antileukocytic animals does not possess any cytotoxic action, but has a growth-stimulating effect comparable to that of embryo extract, if tested on the first passage of leukocytic cultures.

The leukocytes of these rabbits explanted in the rabbits' own human toxic or chicken toxic antileukocytic plasma showed normal growth.

While exposure of antileukocytic serum to a temperature of 56 C. for one hour decreases, but does not destroy, the antileukocytic qualities of the serum, exposure of the serum to ultraviolet rays for the same period has no effect.

#### HISTOLOGY OF THE ANTILEUKOCYTIC RABBITS

Borgi<sup>12</sup> recently reported that rabbits and mice given repeated injections of leukocyte suspensions show histologic changes in the spleen, liver and lung resembling those present in myeloid leukemia. He noticed a marked proliferation of the reticulo-endothelium of these organs and observed myeloid foci in the livers of the mice. The spleens were markedly increased in size, and especially the constituents of the pulp were augmented, while the follicles were unchanged. There was, moreover, a marked erythrophagocytosis, besides pigmentation and leukocytic infiltration of the reticulum. Borgi believed that these changes may possibly be preleukemic.

Histologic examination of five rabbits given repeated injections of human, chicken or beef leukocytes did not show any constant changes that might be attributed to the injections. Except a more or less marked brown pigmentation in the spleen of two animals, no pathologic changes were seen. As such pigmentations occur also not infrequently in otherwise normal rabbits, they cannot be regarded as effects of the injections. There was no evidence of any reticulo-endothelial proliferation in any of the animals examined.

#### CONCLUSIONS

Antileukocytic serum has an antiproliferative and cytolethal effect on leukocytes and other tissue cells of the same species against which the serum has been prepared. Antileukocytic serum has apparently also a slight to moderate antiproliferative effect against leukocytes of a different species. Serum prepared against leukocytes of leukemic chickens is ineffective against human leukemic leukocytes.

# THE EXPERIMENTAL PRODUCTION OF GLOMERULONEPHRITIS IN THE RABBIT\*

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ROCHESTER, MINN.

The study of nephritis induced experimentally in the rabbit by bacterial injections has been complicated by the frequency with which this animal acquires renal lesions spontaneously. In earlier investigations, almost without exception, the lesion found was ascribed to the material injected, and yet, as has been shown most clearly by Bell and Hartzell,<sup>1</sup> all of the lesions described are found spontaneously in the kidney of the rabbit. Spontaneous nephritis in the rabbit is focal, usually beginning at the corticomedullary juncture with an infiltration by lymphocytes which involves the tubules, and which produces, as end-results, wedge-shaped scars with their bases at the surface. In spontaneous nephritis, numerous glomeruli may be found at times in various stages of destruction, from slight thickening of the capsule to complete sclerosis; hyaline changes in the glomerular tufts, in my experience and that of my associates, are not found. In many sclerotic portions, the glomerular tufts appear almost normal, lying in a mass of connective tissue. Another type of lesion that I have observed to occur spontaneously was first called attention to by LeCount and Jackson.<sup>2</sup> There is a wedge-shaped portion, lighter in color than the surrounding tissue, with its base at the surface of the kidney, and appearing, in the gross, opaque yellow, almost like an abscess. On histologic examination, enlarged tubules, lined by a low, atrophic epithelium, are found in this region. The glomeruli are, in the main, largely intact. I had the opportunity to see such a lesion in its acute stage when, at the very tip of the wedge-shaped portion, there was a group of tubules that were plugged with polymorphonuclear exudate, and these tubules, with the changes, could be traced to the tip of the papilla. Upward into the cortex there was an acute intertubular infiltration, which extended only a short distance, and which did not reach the surface of the kidney. These infiltrations are frequently seen in the papilla in the chronic and subacute stages, and they have been a source of difficulty in the interpretation of experimental lesions caused by colon bacilli. I have not

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\* From the Section on Pediatrics, the Mayo Clinic.

1. Bell, E. T., and Hartzell, T. B.: J. Infect. Dis. **24**:628, 1919.

2. LeCount, E. R., and Jackson, Leila: J. Infect. Dis. **15**:389, 1914.

been able to isolate an organism from this type of lesion; cultures of the urine are usually sterile. Exceptionally, I have observed acute glomerulitis occurring spontaneously in rabbits, and resembling closely lesions described by Blackman, Brown and Rake.<sup>3</sup>

Another type of nephritis that apparently occurs spontaneously in the rabbit was observed by Mallory and Parker<sup>4</sup> in a series of four of a group of eleven rabbits. The pathologic process begins with proliferation of the endothelial cells of the glomeruli leading to occlusion of the capillaries. A single loop or an entire glomerulus may be involved. The distribution is uniform; the percentage of involvement of the glomeruli is not stated. This type of spontaneous lesion represents an important difficulty in the use of rabbits for work on experimental glomerulonephritis. It apparently represents the acute stage of the first spontaneous glomerulonephritis described in the rabbit. Jaffe<sup>5</sup> described a single case of glomerulonephritis in the series that he examined in which the lesion was marked by a sclerosis of the glomeruli and was also marked by extensive calcification of the tubules.

A large number of observers have attempted to produce glomerulonephritis in rabbits with various types of streptococci. Bell, Clawson and Hartzell,<sup>6</sup> Longcope,<sup>7</sup> Birkhaug and Howard<sup>8</sup> and LeCount and Jackson<sup>2</sup> were unable to produce characteristic glomerular changes. Others have produced lesions that were, however, indistinguishable from lesions that are now known to occur spontaneously in the rabbit. Duval and Hibbard,<sup>9</sup> in 1926, reported producing glomerulonephritis in the rabbit, but their pictures are not at all convincing, and the repetition of the work by Reith, Warfield and Enzer<sup>10</sup> gave entirely negative results. Kinsella and Sherburne<sup>11</sup> in one instance produced acute changes in the kidney of an animal with an injured aortic valve; the animal survived an intravenous injection of *Streptococcus viridans* for

3. Blackman, S. S.; Brown, J. H., and Rake, Geoffrey: *Bull. Johns Hopkins Hosp.* **48**:74, 1931.

4. Mallory, F. B., and Parker, Frederic, Jr.: *Am. J. Path.* **3**:91, 1927.

5. Jaffe, Rudolf: *Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere: Kaninchen, Meerschweinchen, Ratte, Maus*, Berlin, Julius Springer, 1931.

6. Bell, E. T.; Clawson, B. J., and Hartzell, T. B.: *Am. J. Path.* **1**:247, 1925.

7. Longcope, W. T.: *Bull. Johns Hopkins Hosp.* **45**:335, 1929.

8. Birkhaug, K. E., and Howard, R. P.: *Proc. Soc. Exper. Biol. & Med.* **28**:95, 1930.

9. Duval, C. W., and Hibbard, R. J.: *J. Exper. Med.* **44**:567, 1931.

10. Reith, A. F.; Warfield, L. M., and Enzer, Norbert: *J. Infect. Dis.* **46**:42, 1930.

11. Kinsella, R. A., and Sherburne, C. C.: *Proc. Soc. Exper. Biol. & Med.* **20**:252, 1923.

seventeen days. Rich, Bumstead and Frobisher<sup>12</sup> produced intracapsular hemorrhages in 28 per cent of seventy-nine animals that were given injections of a filtrate of streptococci. Clawson<sup>13</sup> stated that from 4 to 45 per cent of glomeruli contained infarcts, crescents or hyaline lesions when streptococci and particulate material were injected. When only bacteria were injected, not more than 4 per cent of glomeruli of any one animal were involved. In only four of sixteen rabbits were there glomerular changes: in one rabbit crescents were noted; in the other three, only hyalinization of the glomerular tufts. None of the animals was examined later than thirty-six days after injection. Long, Finner and Patchen<sup>14</sup> injected the protein of the bacillus of tuberculosis in doses of from 35 to 100 mg. directly into the renal artery of tuberculous animals, and in this way produced acute, proliferative glomerulonephritis. Blackman, Brown and Rake reported acute and subacute changes in the glomeruli following repeated injections of pneumococcus autolysate. The early changes were hemorrhages in capsules, and the final changes, hemorrhages in tubules. More recently, Lukens and Longcope,<sup>15</sup> by injection of killed cultures of hemolytic streptococci directly into the renal artery of rabbits, were able to produce acute glomerulitis in about 50 per cent of animals that received injections. The lesions were more frequent in sensitized animals than in nonsensitized animals.

#### EXPERIMENTS

My experiments were performed on three groups of rabbits. Animals of the first group were given injections of organisms isolated from the middle ear of a patient in whom nephritis developed. Those of the second group received injections of organisms isolated from the urine of a patient who had acute hemorrhagic nephritis, and those of the third group received injections of green-producing streptococci isolated from patients with subacute bacterial endocarditis.

The organism used in the first group, and isolated at the time of the incision of the ear-drum, was a hemolytic streptococcus. Twenty-four animals were given from one to nine intravenous injections each. The first injection into some of the animals was subcutaneous. At a later date, three animals were given intra-aortic injections. None of the animals that received injections gave any evidence of glomerulonephritis, although many had the usual focal nephritis seen in rabbits. One animal died twenty-four hours after an aortic injection, and many of its glo-

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12. Rich, A. R.; Bumstead, J. H., and Frobisher, Martin, Jr.: *Proc. Soc. Exper. Biol. & Med.* **26**:397, 1929.

13. Clawson, B. J.: *Arch. Path.* **1**:911, 1926.

14. Long, E. R.; Finner, Lucy L., and Patchen, P. J.: *Am. J. Path.* **4**:571, 1928.

15. Lukens, F. D. W., and Longcope, W. T.: *J. Exper. Med.* **53**:511, 1931.

meruli were involved in an acute inflammatory process; in the opposite kidney, however, relatively few glomeruli were involved. One animal lived thirty days after an aortic injection, but there were no changes in its glomeruli.

The organism used in the second group, and isolated from the urine, was a green-producing streptococcus. Eight animals received repeated intravenous and subcutaneous injections. In not a single instance were any glomerular lesions of note found. Sixty-six days was the longest time that an animal in either the first or the second group lived after the first injection.

When the first two series turned out so uniformly negative, I felt that possibly we were expecting changes to occur too soon after inoculation. It was then decided to give the animals injections at monthly intervals with three increasing doses. In the third series of experiments, seven animals were employed. Four of the rabbits received their first injection of streptococci subcutaneously. The twenty-four hour cultures were added to milk to which, just previous to injection, an amount of rennet sufficient to produce coagulation had been added. In this way, a chronic focus was produced. The second and third injections were given intravenously at monthly intervals. To the other three animals all three injections were given intravenously at monthly intervals. Of the animals that received a primary subcutaneous injection, one died on the fifth day after the first injection and a second a few days after the second injection. The death of the second animal occurred during a week-end, and its body was so badly decomposed when examined at necropsy that it was of no value. The two remaining animals were killed, respectively, 328 and 326 days after the first injection.

From the former animal the left kidney was removed, April 25, 1930. May 7, the rabbit was given a subcutaneous injection of a culture, suspended in milk, of green-producing streptococci isolated from a patient with subacute bacterial endocarditis. A local abscess developed, which took about six weeks to heal. The first intravenous injection was of 2 cc. of a twenty-four hour culture; the second, of 10 cc. of a twenty-four hour culture. On Jan. 29, 1931, 267 days after the first injection, a small, wedge-shaped piece was taken out of the right kidney for histologic examination. March 31, the animal was killed. Grossly, the kidney was scarred at the lower pole, and also where the specimen had been removed. Available for histologic study, as a control, were the left kidney, removed before injection, a small wedge of the right kidney removed 267 days after the first injection, and the remainder of the right kidney, removed 328 days after the first injection. The concentration of urea, shortly before death, was 27.9 mg. in each 100 cc. of blood. The urine was negative for albumin, casts and cells, and culture of the urine gave negative results.



Sections of the left kidney, removed before injection, contained practically normal glomeruli. Throughout, an occasional sclerotic glomerulus was found, as is the case in sections from most kidneys. No changes were seen in any glomerular tufts.

In the small piece of right renal cortex removed 267 days after the first injection was found less change than was found in any of three animals concerning which a detailed report of renal changes is given in this paper. This was due, possibly, to the small amount of tissue available for examination. The hyaline degeneration of the glomeruli was slight, but definite adhesions between glomerular tuft and capsule were seen.

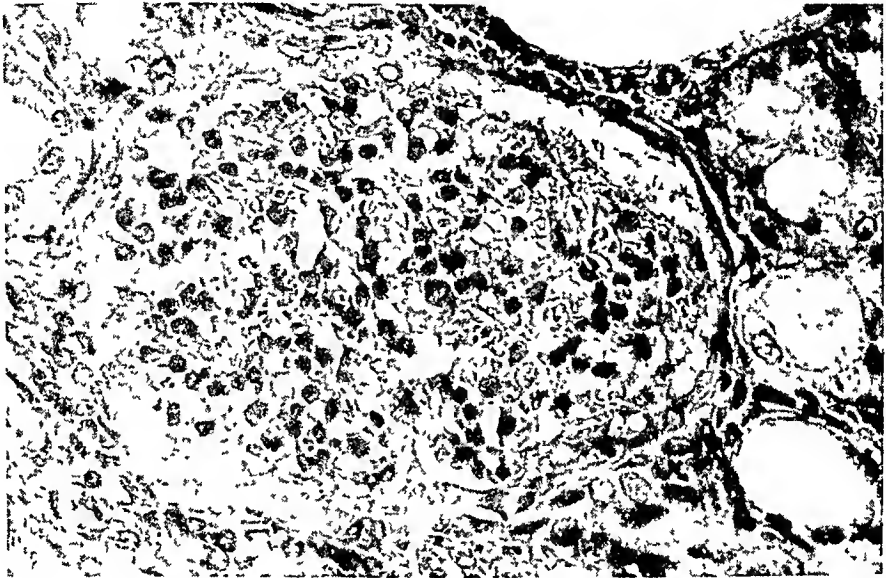


Fig 1—Hyaline material filling capsule.

In the right kidney, removed 328 days after the first injection, were lesions most suggestive of changes seen in glomerular nephritis of the human type. The most acute lesion seen was eosin-stained hyaline material filling the space between a glomerulus and the capsule, and with acute proliferative changes in the capsule (fig. 1). This change could be followed through seven sections of this particular glomerulus. In one of the serial sections, the proliferation resembled very closely the crescent seen in figure 5. This probably represents the early stage of the chronic lesions seen so plentifully in this kidney. The lesion was diffuse, affecting individual loops of a large proportion of the glomeruli, and in some instances a large part of a glomerulus, so that only a few capillaries were still open. Most characteristic were the crescent-like formations which were present in fairly large numbers. Adhesions between the glomerulus and the capsule were frequent when the glo-

merulus did not seem markedly involved. Hyaline change, with obstruction of a small group of glomeruli or capillaries, and adhesions were present in more than 50 per cent of the glomeruli. Formation of



Fig. 2.—Pathologic changes in five of seven glomeruli.

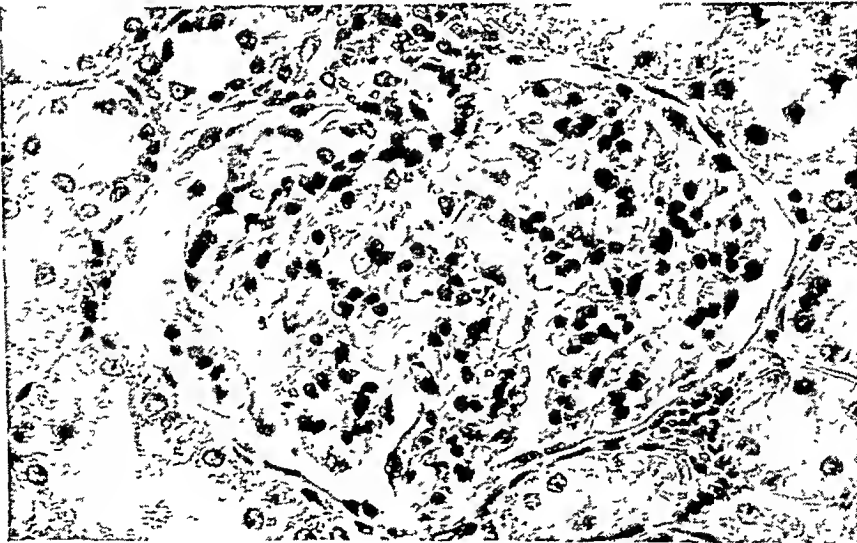


Fig. 3.—Adhesion of glomerulus and capsule.

crescents was considerably less marked than hyalinization, as can be seen best in the low power photomicrograph (fig. 2) of a portion of this kidney. Five of seven glomeruli were markedly involved. In two, there was marked thickening of the capsule, in one of which the local thickening suggested a crescent. In two others were definite

crescents, with adhesions and destruction of a few loops. The fifth illustrated clearly the formation of adhesions between a glomerular loop and Bowman's capsule. The other two of the seven glomeruli, only partly seen in the picture, appeared normal. Several of the glomeruli seen in figure 2 are shown in high magnification to illustrate the changes in detail (figs. 3 and 4). Another typical crescent is pictured in figure 5. Figure 6 represents a section of the left kidney, removed before the injections were begun, showing all glomeruli to be normal. In both kidneys, as well as in the liver and spleen, staining for amyloid gave negative results.

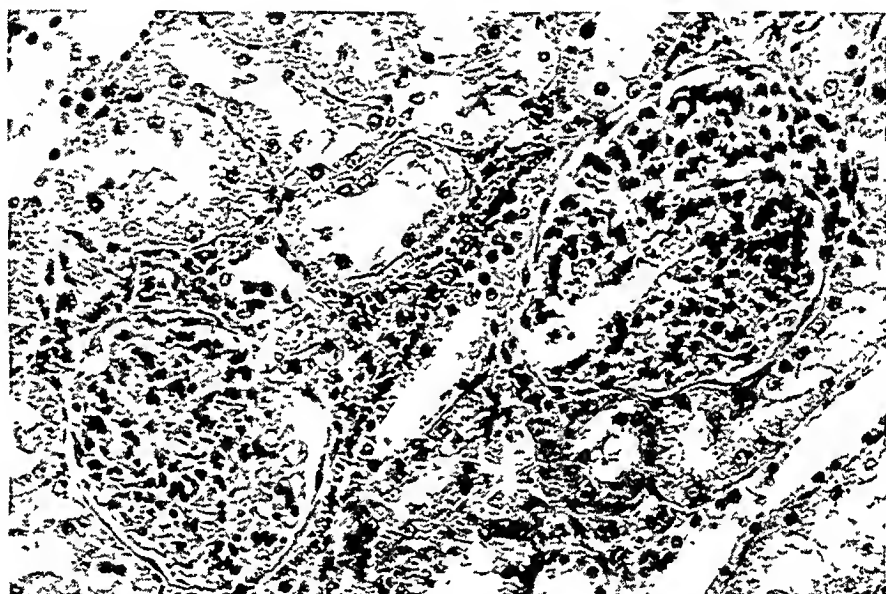


Fig. 4.—Adhesions and crescent.

The latter of the two animals that were killed, respectively, 328 and 326 days after the first injection was subjected to removal of its left kidney, Jan. 16, 1931. Grossly, the kidney appeared normal. March 31, the animal was killed. At necropsy it was found that the animal was pregnant, and that there was a moderate degree of coccidiosis. The kidney did not seem enlarged, and it was sclerotic at its lower pole, where only small islands of normal cortex remained. The upper two thirds of the kidney appeared smooth and of normal color; on section, the relationship between the cortex and medulla appeared normal. The concentration of urea shortly before death was 20.2 mg. in each 100 cc. of blood. The blood culture was negative. Culture of the urine gave negative results at necropsy, but three weeks before a few colon bacilli and many streptococci of green-producing type had been grown from the urine.

Both kidneys were available for study; one had been removed 267 days after the first injection, and the other was obtained at the time the animal was killed. The changes in the two kidneys were essentially

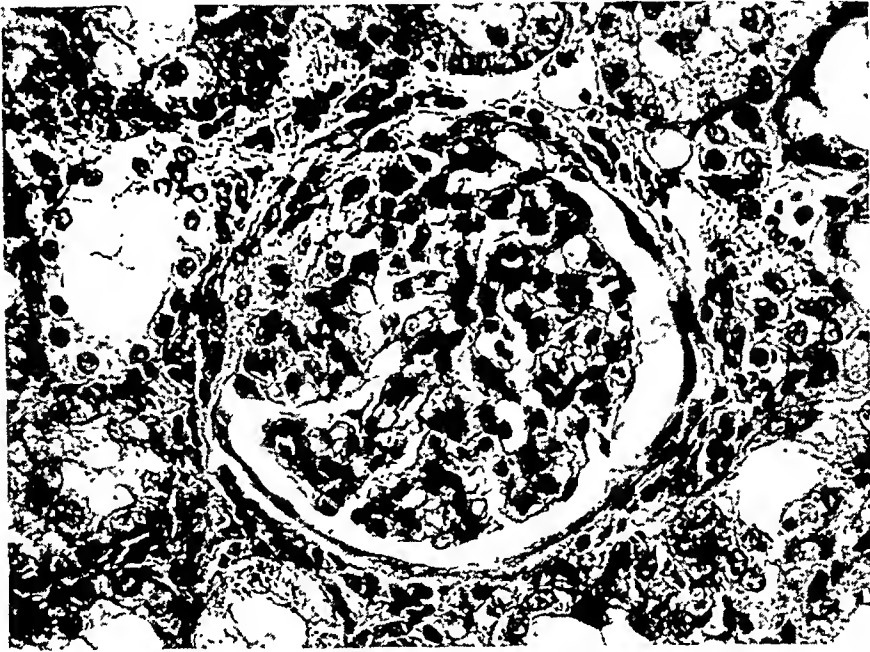


Fig. 5.—Crescent.

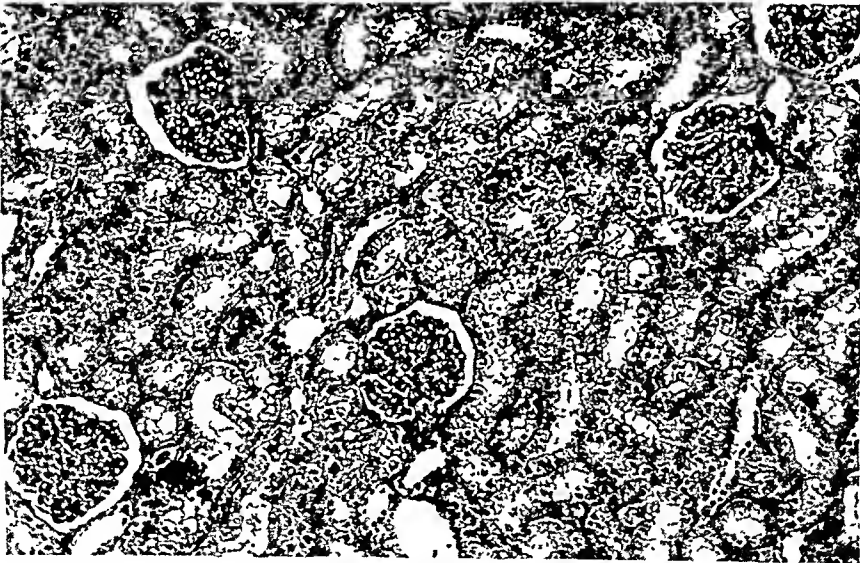


Fig. 6.—Normal glomeruli from left kidney removed before injections.

the same, but the degree of involvement of the glomeruli as shown by the hematoxylin and eosin stain was about 60 per cent for the kidney that was removed in life and 95 per cent for the one that was removed

after death. In the left kidney, removed earlier, the changes were hyaline in nature; a few loops of some glomeruli were involved and almost the entire structure of others. This destruction of the tuft was distinctly brought out with the Mallory-Heidenhain stain, by which the absence of capillaries in the sclerotic, blue-staining portion was revealed. In some glomeruli there was definite evidence of increase in the number of cells, and in some there were signs of nuclear fragmentation. In numerous glomeruli adhesions were seen between the glomerular tuft and the capsule. The material in these hyaline portions took the congo red stain for amyloid. Here, as in the other of these two animals, the stain for amyloid in spleen and liver gave negative results.

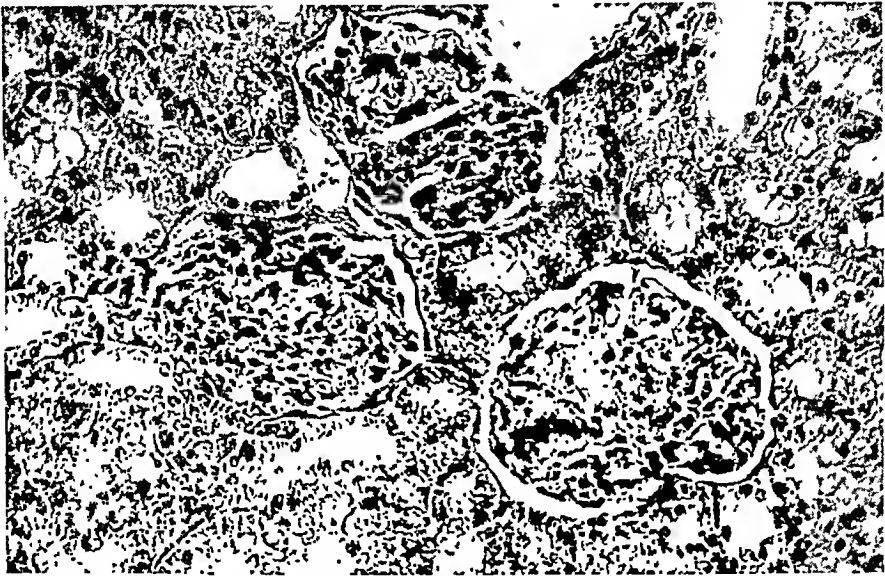


Fig. 7.—Hyaline degeneration of three glomeruli.

The sections taken from the right kidney show not only that more than 95 per cent of the glomeruli were involved, as has been said, but also that the involvement was of definitely more marked degree than that in the left kidney. The crescents were not so typical as in the specimens from the other animal. In figure 7 is a group of glomeruli presenting various degrees of involvement, and figure 8 shows a Mallory-Heidenhain stain of another region, illustrating the focal destruction of capillary loops. It is strange that the value for blood urea was not elevated with this marked involvement of glomeruli. This may be explained by the fact that only parts of most glomerular tufts were involved, and sufficient normal capillaries of most of the affected tufts remained to carry on normal function. The condition of the tubules was difficult to judge; the variation is so great even in

apparently normal organs that I do not feel justified in attributing any of the observed changes to the glomerular changes.

Of the three animals that received all three injections intravenously, the first died one day after the third injection, and changes were not found in its kidneys. The second animal died 165 days, and the third 215 days after its first injection. There were some changes in the glomeruli of the second animal, but because the glomeruli seemed compressed by the tubules, the relationship of glomerulus and capsule was difficult to make out in most parts of the sections. Only occasional small hyaline portions were seen. In some instances, adhesions between glomeruli



Fig. 8.—Mallory-Heidenhain stain of hyaline glomerulus.

and capsule were seen, and considerable sclerosis of many of the glomeruli, as seen in figure 9.

The third animal was the only one of those concerning which a detailed report of renal changes is given to die from natural causes, and the fact that necropsy was done several hours after death probably accounts for some of the changes in the renal tubules. The kidneys were large and smooth on the surface. On cross-section, the normal relationship of cortex and medulla was seen, and there was an opaque yellowish-gray tip of the papilla on both sides. In the other organs, nothing abnormal was seen. A block of tissue for section was taken from the liver, but not from the spleen. On microscopic examination, the tips of the papillae were seen to be necrotic, but the necrosis was



very recent, for the outlines of all of the tubules could still be made out. A definite zone of demarcation, with a zone of leukocytes, was seen in the upper third of the papilla.

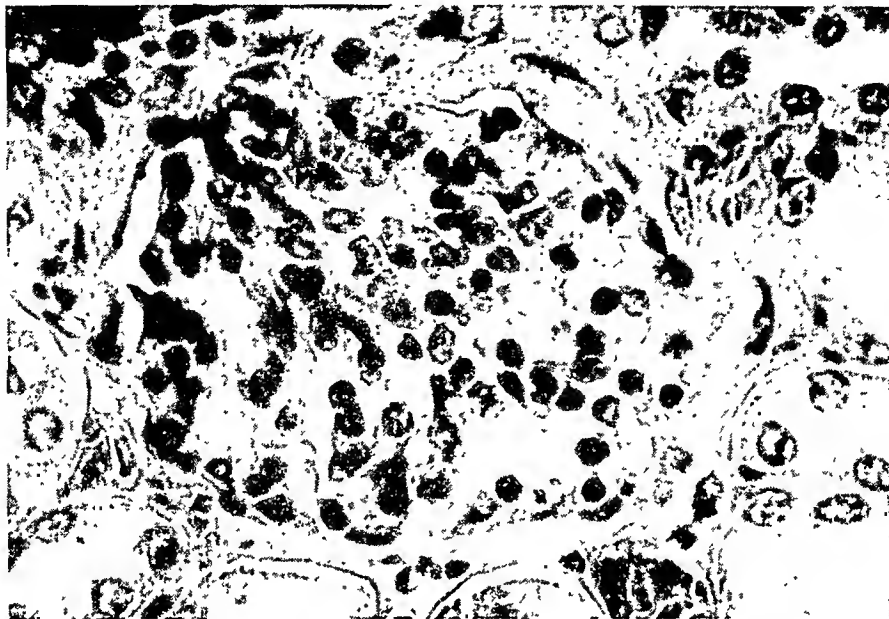


Fig. 9.—Adhesions between thickened capsule and glomeruli.

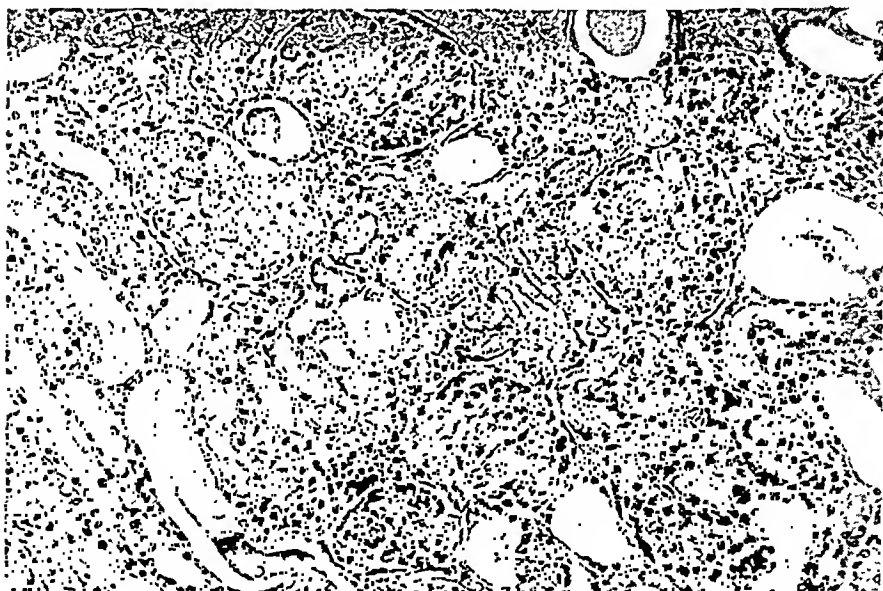


Fig. 10.—Hyaline degeneration of all glomeruli.

The changes in the glomeruli of both kidneys of this third animal were more striking than in any of the other experiments. Every glomerulus was affected; in some were small areas, in others larger areas, of

hyaline degeneration, and in some almost the entire glomerulus was hyaline. There was little cellular infiltration. The capillaries in the glomeruli were few and far between. Definite crescents were not seen, and there was practically no thickening of Bowman's capsule. The histologic picture looked like the final stage of the hyaline changes seen in the animal which was killed after 326 days rather than those seen in the animal which was killed after 328 days, in which crescents and capsular thickening were marked. In this animal, also, the hyaline material in the glomeruli took a definite tint with congo red. The degenerative changes in the tubules were more marked than in any of the other experiments. This may possibly be due to postmortem

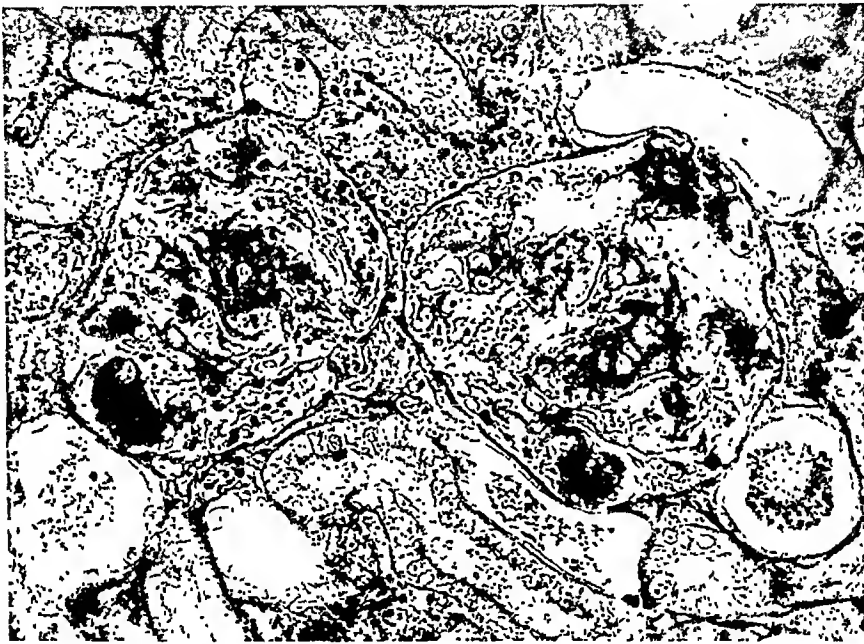


Fig. 11.—Mallory-Heidenhain stain of hyaline glomeruli.

change. Amyloid was not found in the liver. In figure 10 is seen the uniform involvement of all of the glomeruli that appears when they are stained with hematoxylin and eosin, and in figure 11, the focal involvement of the glomerular tufts, when they are stained with the Mallory-Heidenhain stain.

#### COMMENT

Of the seven rabbits in the series to which it was decided to give, at monthly intervals, three increasing doses of green-producing streptococci derived from patients with subacute bacterial endocarditis, five could be examined, and in four of the five glomerular changes were produced. These changes were different from any that previously had been observed to occur spontaneously in the rabbit.



The lesions observed were of such a marked nature, involving, in two of the four animals, more than 95 per cent of all glomeruli, that it was evident that very widespread glomerular involvement had been achieved. It is possible that the changes were due to a hitherto undescribed form of spontaneous glomerular nephritis or that they might have represented a terminal stage of the spontaneous intracapillary glomerulonephritis described by Mallory and Parker. In one of the animals, the glomeruli of one kidney that had been removed before beginning injections were absolutely normal. In the course of the experiment a portion of the remaining kidney was removed, so that concerning this animal, at least, it is possible to say that the kidneys were normal at the beginning of the experiment, and that the glomerular lesions progressed definitely in the sections studied. In one other animal, two stages of the glomerular change could be observed. At the earlier stage, only 50 per cent of the glomeruli were involved; at the later stage, 95 per cent. The older lesions involved a greater portion of the glomerular tufts (95 per cent).

In spite of the fact that one cannot rule out the possibility that the lesions observed represent a type of spontaneous glomerulonephritis hitherto undescribed, it seems likely that the changes are the results of repeated bacterial injection.

# Laboratory Methods and Technical Notes

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## A RAPID AND SIMPLE METHOD FOR MACERATING BONE\*

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Maceration of bone is not widely practiced in hospital laboratories because it is generally believed that the preparation of such specimens is difficult and time consuming. We have been using a simple and rapid method that permits the preparation of specimens of dry bone without the use of elaborate apparatus. Our preparations compare favorably with those obtained with the more complicated methods in vogue in large anatomic and pathologic institutes where steam and defatting tanks are used. The time required for the preparation of a specimen by our technic varies from four to six days. It is best to use fresh, unfixed bone; formaldehyde-fixed specimens can also be macerated, but require a longer period of treatment to insure the removal of the surrounding soft tissues.

### TECHNIC

1. The fresh or formaldehyde-fixed specimen is washed in running water for a few minutes to remove blood, secretions, fixative, etc. After this, as much of the soft tissue as possible is cut away from the bone, but care should be exercised to avoid the too thorough removal of the soft tissues, as the bone or the cartilage may be injured.

2. The specimen is then completely immersed in 10 per cent solution of anti-formin and kept at from 70 to 80 C. for from four to twelve hours. It is preferable to execute this step in an incubator at 80 C., if one is available, as the temperature may be kept more even, and close watching is not necessary. If the specimen is large, it is often necessary to change the fluid after from two to four hours of maceration. The time required for this step depends on the nature of the specimen, the compactness of the bone, the age and the degree of maceration desired. For instance, fetal bones or other bones containing considerable cartilage, the dissolution of which would injure the specimen, necessitate that the temperature be kept at the lower level, and that the length of the treatment should be reduced accordingly. This step is completed when the adherent soft tissue is gluelike in consistency and therefore removable with a stream of warm running water.

3. The specimen is now washed in a steady stream of warm water for about six hours. Should the removal of the soft tissue be incomplete, it may be scraped or brushed off, or removed with a thumb forceps.

4. The bone is now transferred to 5 per cent solution of commercial hydrogen peroxide and kept at room temperature until thoroughly bleached. This usually takes twenty-four hours.

5. Following this, the specimen is dried by exposure to the air (preferably in the sun or on a warm radiator) or in an incubator at 37 C. The bone must be thoroughly dried before it can be defatted.

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\* From the Laboratory Division of the Hospital for Joint Diseases.

6. Finally, the bone is defatted in xylene. The specimen is placed in a closed container and heated at 80 C. for from six to twenty-four hours. If an open flame is used, the container should be immersed in a water bath. An incubator is preferable for the reason stated in step 2. If the bone is very fatty, this step may have to be repeated. In bones of the young, which contain little fat, this step may be omitted. The boiling point of xylene is about 138 C. There is no danger of explosion or fire unless an open flame is applied directly to the xylene, and at 80 C. there is no considerable cloud of xylene vapor. After the bone is defatted it may require an added bleaching as in step 4.



The specimen shows a bone graft in place and fusion of the bodies of the vertebrae and graft.

A bone so prepared allows the study of its external configuration, and when split open, of its internal architecture. Macroscopic deviation from the normal consistency and contour may be easily detected. After experimental operative procedures, the relation and adherence of, for instance, a graft to the underlying bone may be satisfactorily observed in a specimen so prepared. Bone resorption and new bone formation are appreciated when roentgenograms show no significant changes. If histologic examination of the macerated bone is desired, unstained ground disks or unstained frozen sections decalcified by the von Ebner method may be made.

# General Review

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## BIOPSY IN TUMORS\*

C. ALEXANDER HELLWIG, M.D.

WICHITA, KAN.

### HISTORY OF BIOPSY

Every generation has its investigative fashions, which run more or less in the grooves of least resistance or of great promise. Today the morphologic method has lost its leadership in the progress of medical science. The pathologic histology, so eagerly begun one hundred years ago and regarded until the last decade of the nineteenth century as the supreme, if not the only, principle of recognition of diseases, is often derided during the present era of biochemistry and biophysics.

It is true that the morphologic study of cancer produced no cure and uncovered no etiologic agent, but one must not overlook the fact that it laid the foundation on which modern tumor diagnosis and treatment rely. By concluding from his microscopic observations that malignant disease in its first stage is a purely local condition, Virchow (1854) abolished the therapeutic nihilism of his times, which was based on the conception that cancer is a general "dyscrasia." Only then was early radical operation regarded as a logical treatment which promised a permanent cure. For successful attack, early diagnosis was justly recognized as imperative. Again the microscope provided a diagnostic means, the certainty of which is even today unequaled by any other scientific instrument.

The expectation of a serodiagnosis of cancer was raised by the great discoveries of the bacteriologic era. The morphologic fact that during its curable stage the malignant disease is a localized proliferation of tissue cells seems to deprive one almost entirely of the serologic methods which are so useful in diagnosing diseases that at first are general in the reaction against invading micro-organisms. Fry, with his flocculation reaction, obtained in 1,550 malignant and control cases only 73.3 per cent correct results. Various other serologic methods that have been announced with great acclaim did not better fulfil the great hopes that they aroused. The Boyksen, Freund-Kam-

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\* From the Department of Pathology, St. Francis Hospital.

iner, Abderhalden, Kahn and miostagmin reactions are regarded in the leading cancer clinics of the world as unreliable for diagnosing incipient malignant tumors, because of nonspecificity (Bierich).

When Warburg found that under anaerobic conditions, tumor cells are able to split dextrose into lactic acid, it was supposed for a time that a long sought characteristic difference between the tumor cell and the normal cell had been found, but it was soon discovered that the glycolysis of tumors is no greater than that of certain normal tissue. Efforts to diagnose cancer on the basis of Warburg's discovery were unsuccessful. Lauros, using fresh operative material from 136 cases, demonstrated that carcinomatous tissue showed the same glycolysis under anaerobic and aerobic conditions, but that 20 per cent of the non-carcinomatous material behaved like malignant tumors.

Biophysics dealing with such themes as the hydrogen ion concentration, the refractometric index or the electric potential of the blood of cancer patients offered much of technical interest, but no definite contribution for practical diagnosis, worthy of mention. Only the research by Reding and Slosse, confirmed by Holfelder, on the hydrogen ion concentration in the blood serum of cancerous patients appears somewhat promising. According to them, no cancer occurs with a hydrogen value under  $p_H$  7.37, whereas in cancer values between  $p_H$  7.42 and  $p_H$  7.53 are usually found. But other workers obtained contradictory results.

With the introduction of the achromatic microscope into scientific research, the surgeons urged the employment of the new instrument for clinical purposes. Never was greater confidence placed in a new diagnostic method. After Mueller's classic studies (1838) had revealed that all malignant tumors are composed of groups of cells, the world expected the microscopists to discover the specific cancer cell. Lebert, Hannover and other pathologists responded too eagerly to these temptations and described carefully the morphologic structure of the cancer cell in distinction from the normal tissue cell. From their purely microscopic analysis Lebert and Hannover separated from cancer the so-called cancrioid, or epithelioma, because it did not contain the specific cancer cell. The surgeons, under the leadership of Velpeau, were opposed to this dogmatic standpoint, and in the Académie de Médecine at Paris (1855) Velpeau, the eminent surgeon, violently attacked the advocates of the cancer cell and pronounced the epithelioma cancer, basing his opinion on the clinical observation that metastases occur also in this form of tumor. The enthusiastic confidence placed in microscopic diagnosis of malignant tumors was followed thereafter by a period of greatest mistrust. Owing to the defeat of the French microscopists by Velpeau, the development of microscopic diagnosis as a

clinical method was retarded for many decades, and this time the professional pathologists themselves exercised the most cautious reserve.

Biopsy was already known to the famous Danish pathologist Hann-  
over in the middle of the last century; in 1847, Kiwisch recommended the microscopic study of curetted material in suspected cases of uterine cancer; Schuh (1851) and Thiersch (1865) employed this method in surgical conditions, but the introduction of biopsy as an indispensable routine method into the clinical laboratory appears to be entirely the work of Ruge (1879).

Studying the surgical specimens in the Woman's Hospital at Berlin, Ruge found that in 13 of 23 specimens of uterine cervix which had been amputated by the experienced gynecologist Schroeder, the clinical diagnosis of cancer was proved by histologic examination to be erroneous. He therefore announced that the only reliable diagnostic method in doubtful lesions of the cervix is the microscopic study of biopsy material. In 1881 he required diagnostic curettage in suspected cases of cancer of the uterine body, as the only means for early diagnosis. Ruge described the histologic differential diagnosis between glandular endometritis, atrophy of the mucosa and sarcoma of the uterus and pointed out that the malignant adenoma is only a special form of uterine cancer. He urged recognition of this type of cancer in curetted material, from its atypical cell form and arrangement, before its destructive properties were manifest. The professional German pathologists raised their voices not only against the microscopic diagnosis of malignant adenoma, but of any malignant tumor in curetted material, and maintained that since cancer is a destructive epithelial growth, a certain histologic diagnosis would be impossible before invasion of the deeper structures and metastases were evident. They derided Ruge's so-called "Stueckchendiagnose," and failing in this diagnostic method by lack of experience and by unfamiliarity with the special anatomic conditions of the female organs, they preserved a lamentable indifference.

As late as in 1888, Virchow himself in an authoritative article emphasized the uncertainty of biopsy and warned his confrères to respond to the clinicians' demand, fearing that only disillusionment would result, comparable to the pitfall of the French microscopists in 1855. His pessimistic opinion was possibly somewhat influenced by the tragic rôle that biopsy had played in the disease of the second German emperor. Three specimens of tissue which were excised at different times from a laryngeal tumor of this distinguished patient had been submitted to Virchow, and on all three occasions this great pathologist had failed to recognize the true nature from the microscopic study, while the surgeon von Bergmann had made the diagnosis of cancer on his first clinical examination. Virchow was convinced

that real knowledge of the malignant disease process can be acquired only from the postmortem room, and during the last decades of the nineteenth century the professional pathologists were occupied with the detailed study of the morphology of tumors, the separation of varieties of the disease and the elucidation of histogenesis. Invaluable as these painstaking studies were, it cannot be denied that the activity of the pathologists in their special institutes brought forward a deplorable isolation of pathologic anatomy from clinical research and practice. Ruge therefore, in 1888, warned the clinicians not to wait for help from the professors of pathology, but to develop the art of biopsy independently by microscopic investigation in their particular field. Ruge's own practical results were acclaimed by the scientific world in the following years. Biopsy material was sent to him even from foreign countries, and his diagnostic method finally was accepted by most hospitals, not only in the gynecologic specialty, but also in other surgical branches. In 1889, at the German Surgical Congress, the eminent surgeon von Esmarch emphasized the necessity of microscopic diagnosis in all doubtful cases of tumor before a mutilating operation.

- The somewhat dramatic evolution of modern methods of biopsy is intimately connected with the invention of the freezing microtome. The advantages of immediate microscopic examination during operation, which made the interval of days between diagnostic and final operation unnecessary, were so obvious that many attempts were made to overcome the technical difficulties. Wilson (1905) deserves the credit for the development of the first reliable method to give undistorted, beautifully stained sections in a very short time. The more recent technic by Terry, based on an entirely new principle of supravital superficial staining of razor sections, has passed the experimental stage and is more and more accepted by surgical laboratories.

These rapid microscopic methods which require daily practice, the advent of radiation therapy and the increased knowledge of the variations in different types of cancer made the laboratory diagnosis so highly specialized that in connection with the great surgical clinics of this country a group of surgical pathologists has arisen with continuous experience in the pathology of tumors, of quite a different type from that of the old dead-house pathologist.

#### DANGER OF BIOPSY

The most serious objection to biopsy is that it may not conform with the noble principle of medical art: *Primum non nocere*. It is true that biopsy, being a surgical procedure, cannot be regarded as absolutely harmless. Besides the complications of any operation—hemorrhage, infection, unexpected injury to organs—one must con-

sider the special dangers pertaining to the incision into tumors, i. e., stimulation of growth and dissemination of tumor cells through the blood and lymph vessels.

The solution of this practical problem was attempted in several interesting experiments. Lubarsch used many series of animals with tumors in the hope of ascertaining what effect mechanical forces could exert on the rate of growth. He inoculated sarcoma into mice and rats, traumatized spontaneous tumors in rats and dogs with the forceps and injected homologous and foreign blood over a period of weeks and months, but he was not able to observe any increase in the rate of growth of these tumors. The morphologic structure of the traumatized tumors was unchanged, and there was no increase in mitotic figures. In mice that had two tumors, the traumatized one at times regressed or remained the same size, while the other grew. This most intelligent work of Lubarsch arouses skepticism in regard to the conclusions which Nather drew from his own experiments, that even an interval of a few days between diagnostic and radical operation may be disastrous. This author implanted mouse carcinoma intramuscularly into 30 mice and made biopsies in one half of them. Four days afterwards, the experimental animals were found to weigh about 5 per cent more than before, and Nather believed that this considerable increase in body weight was due solely to an enormous propagation of tumor growth, following diagnostic incision.

The incidence of metastases after biopsy was the object of the experiments of Wood. He inoculated about 400 animals with Flexner rat carcinoma, a growth that normally metastasizes to the lungs of a given strain of rats in approximately 20 per cent of the animals. These 400 animals were divided into two groups. In one of them a slice of tissue was taken out of the tumor, the skin was sewed back over the growth, and at the end of ten days the tumor was excised to prevent further metastasis. At the same time, the tumors of the 200 control animals were also excised. A period of ten days was selected because that was the utmost limit required for the preparation of a microscopic section. Both groups of animals were allowed to live for several months and were then killed. The percentages of the metastases to the lungs in the animals the tumors of which were incised and in the controls were practically the same, showing that, at least in rats and with the use of the Flexner carcinoma, no increased metastasis was caused by a carefully executed biopsy.

Wood and Tyzzer studied another type of mechanical injury to tumors—gentle massage. This experiment does not apply to the amount of compression caused by incision with a sharp knife, but to the much abused clinical method of palpating a tumor. Wood and Tyzzer gently



massaged animal tumors for a few minutes on two or three successive days, then removed the tumors surgically to prevent further metastases and kept the animals a month until the metastases had had a chance to develop. The number of metastases to the lungs was greatly increased after massage, in many instances doubled.

Several investigators studied the influence of traumatism to artificial tar cancer, but the results were not uniform. Deelman observed in the mouse that scarification of the area of the skin that is subjected to the application of tar produces vigorous regeneration of the epidermis and hastens the appearance of cancer. Mertens could not confirm these experiments of Deelman, but by excising the first papillomas caused by tar painting, he observed malignant transformation of the tissue just on the margin of the excision. Deelman's experiments were repeated by Roussy, Leroux and Peyre, but they were not able to shorten the time of production of tar cancer by scarification, nor was the incidence of growth increased by traumatism.

Daels placed sutures saturated with irritant substances under the tarred skin of animals, but the resulting scar formation beneath the skin seemed even to reduce the disposition toward development of cancer.

In the clinical observations suggesting a stimulation of malignant growths by biopsy, there remains only the *post hoc, ergo propter hoc* type of logic. Wood stated that the scientific basis of the opinion that diagnostic incision into tumors is a dangerous procedure usually rests on one or two cases occurring in the experience of the individual. Knox, in her thorough review on trauma and tumors, did not doubt that sarcomas of the extremities are frequently aggravated, symptomatically at least, by superficial injuries. In tumors of the bones and brain it is conceivable that the cellular portions might be in some cases temporarily stimulated by the congestion resulting from a hemorrhage and the subsequent repairing, but the opposite possibility must be kept in mind, that necrosis of a portion of the tumor and shrinkage may be directly due to the injury. Lubarsch pointed out that untraumatized tumors do not grow with any regularity, and that considerable periods of rest may alternate with active periods of growth without apparent cause, a phenomenon that was abundantly studied in the animal tumors by Woglom.

According to Knox, the possibility that benign tumors may be converted into malignant ones under the influence of trauma is apparently remote, although a few types are believed to be more commonly susceptible to such a transformation. It is doubtful whether an acute trauma has ever accomplished such a change. Ewing stated definitely that a single trauma had never in his observation changed a benign,

quiescent remnant of tumor cells into a malignant tumor. It is impossible to attach much importance to the original trauma when the course of the case makes it obvious that the tumor is capable of becoming malignant without trauma. Hosoi reported a case of multiple neurofibromas in which a tumor of the inferior cervical region became sarcomatous about one year after operative intervention. It has been clinically observed that partial removal or any operative trauma may activate a benign neurofibroma into a sarcoma. Furthermore, even after complete extirpation of a sarcomatous tumor another neurofibroma in a different location may undergo sarcomatous transformation (von Winiwarter). In other cases, in spite of repeated operations, no sarcomatous changes occur, and it is still inexplicable why in some cases a malignant transformation supervenes after a single surgical intervention while in other cases even after repeated operations no malignant changes arise. In the 2 cases of malignant transformation of neurofibromas that I observed, no trauma preceded this change.

Ewing, who stated that the removal of a small, carefully selected portion of an accessible tumor seldom results in any harm, regarded biopsy in uterine carcinoma as a not inconsequential matter. Considerable crushing of tissues is usually inflicted in cutting deeply into the indurated cervix, and there is much ground for attributing the high percentage of recurrence of carcinoma of the cervix and the corpus to the mechanical dissemination of tumor cells during diagnostic and final operation. He expressed the fear that energetic curettage would seem more likely to disseminate tumor cells into the lymphatics than to cure even cases strictly limited to the mucosa. Curettage for the diagnosis of carcinoma of the corpus was therefore regarded by him on anatomic grounds as distinctly dangerous.

Pauchet observed perforation by the curet in a case of suspected uterine cancer in a 60 year old patient. The perforation healed spontaneously, but at the radical operation, which was undertaken one year later, the growth had broken through the wall and invaded the ileum. Pauchet stated that, since that time, he had performed the radical operation without previous biopsy in any woman who had passed the menopause, on clinical suspicion. Also Ludwig emphasized the danger of diagnostic excision and curettage in uterine cancer. He saw generalization of apparently radically removed small tumors, which must be attributed to the opening of lymph and blood vessels during biopsy. Norris expressed the belief that metastasis by transtubal migration of cancer cells is probably frequent after diagnostic curettage. As evidence of this occurrence he pointed to the fact that in his 101 cases of carcinoma of the uterine corpus there were eight instances in which ovarian involvement was present. Still he did not take this as an argu-

ment against diagnostic curettage. He emphasized that carcinoma of the fundus in its early stage is often obscure, and that the surgeon's choice rests between waiting for the development of the more pronounced symptoms and hysterectomy. Diagnostic curettage is therefore the lesser evil. As a matter of fact, Norris' best three year results were secured in those patients in whom diagnostic curettage was performed and can probably be explained by the fact that these patients as a group were operated on much earlier. Bloodgood stated that in practically all cases of early cancer of the uterine cervix and corpus in which a diagnosis had been made by curettage or by removal of a piece of the cervix, there had always been an interval of time—days or weeks—and that no one had yet been able to detect the dangerous factor, if present. Bloodgood's statement is verified by the experience of Martzloff. In a series of 38 patients living and well many years after operation for carcinoma of the cervix, he found that 36.8 per cent were subjected to diagnostic curettage several days before the radical operation. This procedure evidently did not jeopardize them.

Frank strongly condemned the tendency to remove the uterus on suspicion of malignancy, based on the theory—as yet unproved—that excision of cervical specimens and exploratory curettage of the cervix and fundus uteri are prone to disseminate cancerous infection; likewise, he condemned hysterectomy in cases in which curettage failed to disclose the cancer. The question arises whether the mortality due to complete hysterectomy in skilled hands does not far exceed the problematic prophylactic gain. According to Stierlin, it is doubtful whether biopsy in uterine cancer has ever led to increased rapidity of growth and dissemination of malignant cells. Adler, Vogt, Lahm and Meyer, whose biopsy material from gynecologic patients exceeds 2,000 per annum, all have regarded diagnostic excision and curettage as of small inconvenience to the patient, and even an interval of several days between diagnostic and final operation is in their opinion harmless. And Novak asserted that even if there were some risk, he would resort to biopsy in the group of cases in which the diagnosis cannot be made in any other way, since the information to be gained is of such vital importance to the patient that it far more than counterbalances any supposed or real danger of biopsy.

The most exhaustive study of biopsy in tumors of the breast was made by Bloodgood. When a distinctly malignant tumor was excised with a good margin and the wound closed without thermal or chemical cauterization, and an interval of from two weeks to two months passed before the complete operation, the five year cures were reduced to 10 percent, whether the lymph glands were involved or not. Bloodgood's studies, which have been extended continuously for twenty-five years,

show clearly that there is danger in excising a cancer of the breast without cauterization and waiting longer than two weeks for the radical operation, as indicated by the microscopic study. Bloodgood observed no difference in five year cures with and without biopsy, if chemical or thermal cauterization was employed. From his vast experience he concluded that it is safer for the patient if the surgeon who is not prepared for frozen sections in the operating room performs the complete operation when the clinical picture and the gross appearances at the exploratory excision suggest malignancy. The same opinion was held by Lee, Kuettner and Krecke. Halsted from his long clinical experience feared biopsy and would rarely allow an interval of time for microscopic study. Welch agreed with Halsted that at least on theoretical grounds there is danger in biopsy, especially if there is a longer interval between diagnostic and final operation. Wintz, basing his figures on a large amount of material, emphasized that biopsy in tumors of the breast will double the metastases through the blood vessels and Klose reported cases in which a single aspiration of a carcinomatous cyst stimulated the tumor growth tremendously.

Ewing urged caution in performing biopsies in tumors of the bone, because the incision of encapsulated malignant tumors growing under pressure is nearly always harmful and may be disastrous. With fungating sarcomas, the excision of a portion of tumor tissue is usually accomplished with precision. Ewing pointed to the fact that in some cases in the American Registry of Bone Sarcoma a cure was obtained by amputation after one or even two liberal biopsies, but that in many more the patients died. Bloodgood, on the other hand, stated that there are not enough patients with sarcoma of the bone living five years after operation to estimate whether biopsy previous to amputation or resection adds to the danger of metastasis. He called attention to the fair percentage of five year cures in which biopsy had been performed. One of his 2 patients with periosteal sarcoma, living and well seven years after amputation, had a diagnostic excision with an interval of about two weeks, and among 12 patients with periosteal and diffuse sarcoma of bone who survived the five year period, 2 were subjected to biopsy with an interval of a few weeks before amputation. However, in all of Bloodgood's cases of myxomatous tumors of bone, explored and removed piecemeal, there has ultimately been death from metastases. In those subjected to resection and amputation without this previous piecemeal removal, the patients have remained well. He regarded biopsy, therefore, as dangerous in myxoma, whether of the central or of the periosteal type. In the six years to 1931, Bloodgood had performed biopsy on practically every type of benign and malignant lesion of bone, whether central, periosteal or diffuse, in order to decide from microscopic study whether the tumor was malignant and

if so to proceed immediately with the resection or amputation. Still he recommended that the roentgenologist should be given an opportunity to submit doubtful pictures of lesions of bone to other, more experienced diagnosticians before any biopsy is undertaken by the surgeon. Furthermore, he advised that at least one full course of treatment with the x-rays should be given before biopsy and while submitting the x-ray films to consultants.

Bloodgood's standpoint is based on the well recognized fact that biopsy has some elements of danger, especially when the wound is closed after diagnostic incision and an interval allowed before the radical operation is performed. In biopsy of tumors of bone, Bloodgood's method is to apply the Esmarch bandage and divide the tumor tissue with the electric cautery; in addition, the whole exposed wound is chemically cauterized. If immediate diagnosis with the frozen section method is not possible, the delay until the radical operation should not exceed forty-eight hours. Bloodgood concluded from his unique experience that the evidence against badly performed biopsy is sufficient to condemn it, but that properly performed, it is justified as a last resort.

Kolodny expressed a fear of acceleration of growth not only after an exploratory incision, but even after aspiration of tumors of bone. He did not agree with Bloodgood that cauterization of the operative wound neutralizes the dangers of a probatory incision, since the irritation of the escharotic combined with the surgical insult may increase the rate of growth. Copeland and Geschickter expressed the belief that biopsy does not affect necessarily the prognosis of Ewing's sarcoma, if radical operation or irradiation follows exploration. In 2 of their 8 patients who lived over five years, exploration was done before the radical operation was resorted to. In a case in which diagnostic curettage was followed by irradiation, the patient was well over four years after the treatment. In 6 cases, on the other hand, in which the exploratory operation was performed without further treatment, death occurred in from one to twenty-two months.

Not less uniform are the opinions of experienced German surgeons on the danger of biopsy in tumors of bone. While Mueller, Oehlecker and Schoene reported that they employ this diagnostic procedure in all doubtful lesions of bone without hesitation, Luetge and Baumecker regarded it as absolutely contraindicated in periosteal sarcomas. Holfelder, who favors radiation therapy in all bone sarcomas, waits from three to four weeks after the first x-ray treatment, before he makes a diagnostic excision and believes that under these precautions, dissemination of tumor cells is not to be feared.

The interesting case of osteogenic sarcoma of the humerus which Morton reported illustrates the difficulty of the question whether metastases in a given case must be attributed to a previous biopsy.

The tumor in Morton's case had already broken through the periosteum and invaded the muscles of the arm at the time of the diagnostic incision. In view of the many vascular channels that must have been accessible to tumor cells detached during biopsy, it seems remarkable that at necropsy only a single metastatic tumor was found in the lung. The small size of this nodule suggested implantation at a much later time than that of the biopsy, when the rapidity of growth of the mother tumor is considered. The observation, made long ago by M. B. Schmidt on necropsy material and confirmed recently by Wood in experiments on rats, that many tumor cells carried by the blood stream to the lungs do not develop into metastases, makes the problem of dissemination of tumor cells by diagnostic incisions all the more complex.

Superficial elevated and ulcerating tumors of the skin may, in Ewing's opinion, be safely subjected to the trauma of incision. The establishment of the exact nature of the lesion is far more important to the patient than the inconvenience of a slight operation. Pigmented moles and suspected cases of melanoma, however, should not be touched except by liberal incision. Small rodent ulcers are also extremely dangerous when narrowly excised. According to Bloodgood, in small lesions of the skin—warts, moles, areas of keratosis, small ulcers and nevi—complete excision giving the local lesion a sufficient margin for safety, should cancer be present, is the operation of choice, and biopsy is not necessary. In those cases in which the local process of the skin is so extensive as to make its complete removal a mutilating procedure, a piece must be excised for microscopic diagnosis, which is done in Bloodgood's clinic by the rapid frozen section method. In former years there was an interval sometimes of days between the diagnostic excision and radical operation, but it is difficult to decide whether in these cases the biopsy could account for a certain percentage of recurrences and metastases, since the cancers were all extensive late growths. If a surgeon has to send the biopsy specimen away to a pathologist in another place, Bloodgood advised that the excision be made with the cautery, and that the surgeon try to obtain a report within forty-eight hours. Delbanco and Unna emphasized that a delay of even a few hours is extremely hazardous.

Incision into melanomas for diagnostic purposes is regarded as contraindicated by many surgeons (Baumecker, Luetge). But according to Holfelder, melanomas respond with wide general dissemination not only to biopsy, but even to wide excision. In Broder's 38 cases, in spite of apparently radical removal, 63 per cent of the patients died after the first year, and the average duration of life was only eleven months. In a tumor with such a poor prognosis, the etiologic association of biopsy and metastases will never be convincingly established. Kuettner emphasized that probably most of the metastases observed

after excision of a melanoma originate from minute tumor nodules that are already present in the surrounding tissue at the time of operation. This explanation is substantiated by Lexer's microscopic studies.

In laryngeal tumors biopsy plays a very important rôle. Ewing stated that he had never seen an aggravation of malignant tumors following this procedure, but that several sections are sometimes required to locate the tumor. Brown and Mackenzie, however, expressed the fear that biopsy may convert a benign laryngeal tumor into a malignant one, an opinion that was rejected by Semon's statistical studies. Also Sorensen had never observed in his large material a malignant transformation or an aggravation of growth that could be attributed to the diagnostic excision. According to Bloodgood, in all early lesions of the larynx biopsy is essential. The excised piece is as a rule so small that paraffin sections have to be employed. That a longer interval between biopsy and radical operation may have an element of danger seems apparent from Halsted's cases, in which the delay was at least two weeks. The pathologic examination of the specimens showed early lesions, but all cases recurred in the glands, and the patients died ultimately of cancer. Crawford, on the other hand, examined a number of larynges removed by Lewis and could not find any metastases as the result of biopsy, although in many instances the patient had refused laryngectomy for several months following a positive report from biopsy. Also Jackson had never seen ill effects due to diagnostic excision of laryngeal, bronchial or esophageal tumors, and he asserted that metastases cannot travel far in eighteen hours, which is all the time required for a histologic examination.

In small lesions of the lip which can be radically removed by V-shaped excision there is never any necessity for biopsy (Bloodgood, Ewing), and it makes little difference whether such a lesion is benign or malignant; no local recurrence will be observed. On the other hand, when the process of the lower lip is so advanced that its radical removal would indicate a plastic operation, biopsy must be employed. Bloodgood reported in favor of excision with the cautery and the immediate diagnosis made on frozen sections.

When lesions of the mucous membrane of the mouth, tongue and lip are carcinomatous and still favorable for cure, they are so small that their radical removal with the cautery will never be mutilating (Bloodgood). When the diseased area anywhere in the oral cavity is so extensive that its radical removal would mean mutilation, the chances of a cure are less than 10 per cent, and there is no evidence that biopsy properly performed would reduce this percentage. There are conditions in the mouth that are not carcinoma which resemble the malignant lesion, and for this reason in some cases the most expert cancer specialists must resort to biopsy. Discordant views on the safety of diagnostic

incisions in tumors of the mouth are found in the German literature. Luetttge observed an aggravation of carcinoma of the lip after taking tissue for biopsy, but regarded the taking of tissue from the buccal cavity for biopsy as a safe procedure. Krecke, Bruening and Batzdorf, however, pointed to the danger of this in lesions of the mouth as an established fact, while Heidrich, who had taken specimens in this region for biopsy frequently, and Baumecker did not remember ill effects. Pfahler reported giving radiation treatment either before or immediately after diagnostic excision and continuing the irradiation until the lesion was either found benign by the pathologist or until the curative dosage had been given. Under such management Pfahler never had seen stimulation of growth.

In tumors of the esophagus, biopsy does not seem to add to the dangers of dissemination of the disease; Ewing, Jackson and Batzdorf said that they never had seen any harm even after repeated biopsies on the same patient. There is apparently also no danger in removing small fragments from the rectum through the rectoscope. One of Batzdorf's patients was well seven years after operation in spite of several diagnostic excisions. According to Ewing, the character of polypoid or ulcerating tumors of the rectum may be safely determined from portions of the tissue removed through the speculum or proctoscope, but incisions into hard cancerous strictures were to be avoided.

Malignant tumors of the bladder respond—in Batzdorf's opinion—to the injury of diagnostic excision with rapid dissemination of cells, and in Ewing's opinion it is undesirable to risk extensive incisions from carcinomas of the bladder for microscopic purposes, while Ascher expressed himself in favor of biopsy in all doubtful lesions of the bladder and had not seen any ill effects when the edges of the wound were sealed with the cautery.

Ewing had not learned of any unpleasant results from biopsy in Hodgkin's disease, endothelioma or other tumors of the lymph nodes. On the other hand, incisions into an infiltrating lymphosarcoma are to be avoided under practically all circumstances (Ewing, Juengling, Lazarus, Baumecker). According to Luetttge, the excision of a metastatic lymph gland in lymphosarcoma is not dangerous, and Schnitzler saw a complete disappearance of a lymphosarcomatous growth after biopsy.

Diagnostic aspiration of doubtful thyroid neoplasms was condemned by Bircher, Albert and Socin, since they observed fungation of the tumor in the puncture canal. Ehrhardt, von Eiselsberg, Breitner and Walton regarded diagnostic incision into malignant goiters as unsafe; Klose and Hellwig stated that they use it only when it can be followed immediately by the radical operation. The clinics of Sudeck (Schaedel) and Kuettner (Barthels) recommended biopsy also in cases of inoper-



able thyroid tumor to determine the radiosensitivity, and their end-results have not been unfavorably affected by this procedure. In operable, well encapsulated thyroid tumors of unknown nature, the radical excision of the affected lobe is of course the operation of choice; therefore, in these cases biopsy is unnecessary (Kocher, Socin, Bloodgood, Hertzler).

There are so few cases on record of infection and hemorrhage following biopsy that the inference may be drawn that they are exceptional accidents. The bacteriologic studies of Vinzent and Monod in ulcerating uterine cervical cancer, however, make one wonder why infections do not occur more frequently in this region. In 150 cases of uterine carcinoma, only 9.5 per cent of the aerobic and 12.5 per cent of the anaerobic cultures remained sterile. In all the remaining cultures, spirilla, fusiform bacilli, diptheria-like bacilli, staphylococci, and streptococci, especially hemolytic forms, grew.

As a "rare complication of biopsy in cervix carcinoma" von Steinbuechel reported a case of severe infection, in which the etiology seems convincingly established. Following a diagnostic excision under the strictest aseptic precautions and the suture of the wound, a foudroyant septicemia developed, which resulted after four weeks in the death of the patient. The author attributed the infection to the suture of the biopsy wound. Also Schallehn lost a patient with septicemia five days after careful excision of a piece for microscopic examination in cervical cancer, and he warned against the practice of performing biopsies in the office. It should be done by the surgeon himself in the operating room where immediate diagnosis can be made from frozen sections and the radical operation—if necessary—follow under the same anesthetic. Hoehne gave the same advice, since he observed, after biopsy, parametritis so severe that the radical operation had to be postponed or was made even impossible. Heynemann noticed, in several instances, fever and inflammation of the regional lymph glands after diagnostic incision into cervical cancer, and 3 of his patients who had been subjected to biopsy previous to the hysterectomy died of general pyogenic infection.

In other regions of the body, infection seems to play a much smaller part. Krecke removed a cancer of the breast, which had been incised two days before by another physician for diagnostic purposes; the patient died of septicemia—the only fatal outcome which this experienced surgeon observed among hundreds of amputations of the breast.

Infection is to be feared, according to Ewing, in biopsies in which the incision opens the unbroken skin, the chief protection against infection. It is especially to be avoided in sarcomas of bone, muscle, fascia and lymph glands. Particularly unfortunate results may occur, in Ewing's opinion, when an incision of the skin is followed by deep dissection in the effort to reach an ill-defined and inaccessible tumor.

He remembered disastrous infection and uncontrollable hemorrhage following deep diagnostic incisions into tumors of bone and lymphosarcomas. Kolodny was convinced that the danger of infection after biopsy is especially great in giant cell tumors of bone.

Nather expressed the belief that all dangers of biopsy can almost certainly be avoided if this procedure will be no more regarded as a minor operation which can be easily done in office practice. He advised performing it in the operating room under the strictest aseptic precautions and with preparations to proceed immediately with the radical operation, should the frozen sections reveal malignancy.

#### TECHNIC OF EXAMINING BIOPSY TISSUE

With the construction of the perfected freezing microtome, attempts were made to employ microscopic sections of doubtful tumors during operation and to avoid the unpleasant, if not dangerous, delay between the diagnostic and the final operation. In 1891 Welch made a frozen section of a tumor of the breast at Johns Hopkins Hospital, but Halsted had completed his operation on clinical judgment before the microscopic diagnosis was finished.

It was not until Wilson in 1905 brought out his method of sectioning fresh tissue with the freezing microtome and staining it with polychrome methylene blue that diagnosis during operation became a routine procedure. This method has been tested in the Mayo Clinic during the last twenty-five years, on more than 208,255 surgical specimens removed at operation or exploration, including more than 28,000 carcinomas. It was found that 57 per cent of all surgical cases presented material for microscopic examination, and that 12.6 per cent of all cases required special pathologic consultation during operation. In 2.2 per cent of all surgical cases, the diagnosis and the therapeutic procedure were changed as a result of the pathologic examination.

W. J. Mayo called the evolution of the fresh frozen section dramatic. He stressed the great benefit to the patient of making an immediate histologic diagnosis. The surgeon, knowing the microscopic nature of the pathologic process during operation, has a much better conception of the life expectancy of his patient and is guided in his choice of a radical or of a palliative operation.

Besides Wilson and MacCarty at the Mayo Clinic, Bloodgood deserves the greatest credit for introducing the microscopic diagnosis during operation as a routine procedure. In his numerous papers, he emphasized the necessity of using fresh frozen sections. He expressed himself as confident that such a diagnosis is at least equal in accuracy to that made later from carefully prepared paraffin sections. To increase accuracy and diminish the element of error, the diagnosis of

tissue in the operating room by any rapid method should depend on its routine employment and not only on its use in emergency. Bloodgood stated that the true morphology of the cells is better pictured in the frozen section of the unhardened tissue than in tissue previously fixed in hot formaldehyde and dehydrated in alcohol. He saw the time not far distant when diagnosis from tissue in the operating room will be forced on pathologists. Surgeons are beginning to realize that they are called on to recognize and treat cancer in its microscopic stage. The microscopic section made by these rapid methods in the operating room can be employed not only to diagnose the local lesion, but most accurately to determine the margin that should be given for a benign and for a malignant tumor and to ascertain whether glands near the local lesion which are exposed at the operation show metastases. After having excised the malignant lesion, one can not only study the margin removed, but take bits of tissue from the surface and margin left behind (Bloodgood, MacCarty).

The Committee on the Treatment of Malignant Diseases of the American College of Surgeons endorses the rapid microscopic methods by the following advice: In order that in patients with cancer the possibility of cure shall not be jeopardized, an exploratory operation should be conducted only under such conditions that the appropriate treatment, whether by surgery or by radiation, may be carried out immediately when the diagnosis is established by the pathologists by means of frozen sections.

In European clinics, the use of frozen sections during operation is not as widespread as in this country. There seem to be several reasons for this. The pathologic institute of the larger continental hospitals is as a rule far away from the surgical department, and the pathologist is usually so busy that he has no time to go to the operating room and cooperate personally with the surgeon, in arriving at a histologic diagnosis during operation. Therefore the specimens removed at operation are mostly diagnosed by a surgical assistant who has had one or two years of pathologic training, but who is of course more interested in the technical side of surgery. He leaves the rotating service in the surgical pathologic laboratory before he has acquired a wider experience, which is indispensable, especially for the diagnosis of frozen sections. This present custom of burdening a young surgical assistant with the responsibility of diagnosing the specimens was criticized recently by R. Meyer, who holds the only full-time position of surgical pathologist in Germany and is the successor to C. Ruge, the father of modern biopsy.

The favored rapid method used in German clinics (von Eiselsberg, Payr, Laewen) is that of Walz. He devised it as a substitute for paraffin sections in the hectic post-war days (1919) when the customary

strikes of the gas and electric factory workers made it impossible to rely on a constant temperature of the paraffin oven. After fixing the specimen in hot formaldehyde for one minute, frozen sections are cut and stained with hematoxylin and eosin. For immediate diagnosis during operation, the staining, dehydration and clearing have to be hurried, so that frequently very poor pictures are obtained. In my experience these poorly differentiated and distorted sections cannot compare with the supravital beautifully stained preparations made by Wilson's technic. The latter method enables one to see the intact cell, uninjured by fixation and dehydration, while the cells in Walz's sections are often as shrunken and as different from the living cell as is the raisin from the grape (Cushing). In Wilson's method the sections are handled in solutions designed to preserve the cells as nearly as possible as they were in the living body, and the supravital staining of the cells is so perfect that they show fine nuclear detail distinctly under the highest powers of the microscope.

In appreciating the advantages of rapid sections, some authoritative pathologists have been as reserved as the surgeons have been enthusiastic. Ewing held that with the modern improvements in technic the frozen section often furnishes a prompt and trustworthy decision, but that when the structure of the tumor is atypical, more time should be given for a deliberate study. Occasionally it is of decided value, but often it encourages hasty conclusions and readily leads to error. When the gross appearance leaves doubt, the frozen section usually strengthens the doubt, and it may be distinctly misleading when it suggests a conclusion contrary to the gross diagnosis. Having made more errors by the use of the frozen section method in cases of cancer of the breast than by the gross examination, Ewing had not, he said, resorted to frozen sections in this field for many years, but had relied entirely on gross inspection. No aid from frozen sections can replace the capacity to recognize cancer by sight and touch. Plaut did not trust the diagnosis from rapid microscopic sections either, especially in the field of gynecologic pathology. Reiman said that the "very quick, five minute fix, cut and stain diagnosis" is looked on with suspicion by every good pathologist.

Primrose held that rapid section at the time of operation is by no means a safe and conservative procedure, and that the faith some persons put in these methods is badly placed. According to Sternberg, the rapid method is often unreliable, especially in borderline cases in which an exact microscopic diagnosis would be essential during operation. Sternberg said that, called to the operating room for consultation, he feels embarrassed when the relatives of the patient watch anxiously every step of the frozen section procedure, and that for difficult cases he prefers a deliberate study in the quiet solitude of his

laboratory. Also in Dietrich's opinion, immediate microscopic diagnosis during operation has a limited field.

Neither was Warthin sympathetic toward the "rapid-fire" frozen section method of diagnosis, the universal application of which he regarded only as a fad or a pose. In practice, in his experience, the number of cases requiring diagnosis while the patient is on the operating table is small. For these, he agreed the frozen section method serves a most useful purpose, but he was opposed to its routine use, because no serial sections can be obtained and the staining methods are limited usually to one that is not permanent. He said that he employs as a routine his rapid over-night paraffin method, which permits a microscopic diagnosis twenty-four hours after removal of the surgical specimen.

In regard to the accuracy of the macroscopic diagnosis, MacCarty, with a practical experience very likely unsurpassed by anybody's else, stated that his ability to diagnose gross material from all sources is not more than 81.8 per cent. He determined this figure by actual test in 47,434 surgical and diagnostic specimens. In other words, 18.2 per cent of all specimens, in his experience, require microscopic examination before the diagnosis can be accurately made. This percentage varies with different portions of the body; with the breast it is from 6 to 10 per cent, while with many other regions it is much higher and with some much lower. One can hardly pick out suspicious areas without some ability in the diagnosis of gross specimens, which ability should become greater the more often it is checked by microscopic study. In my last series of 368 biopsies a correct macroscopic diagnosis was made in only 63.6 per cent. The large number of diagnostic curettages in my material may account somewhat for this low figure of correct diagnoses from the gross appearance. In tumors of the breast, for instance, my macroscopic diagnosis checked with that made from paraffin sections in 92.1 per cent.

In the controversy—which is still going on in the literature—regarding the usefulness of immediate microscopic diagnosis during operation, I agree with Wood that any general acceptance or condemnation cannot be made, and that everything depends on the intelligence of the pathologist and the breadth of his experience. If—in cases in which the rapid method does not permit a clearcut diagnosis—the pathologist has the courage to say that he does not know what the tumor is, no surgeon will be misled. If a positive diagnosis cannot be made from the frozen section, either more material should be obtained or the clinician should go ahead on clinical evidence. Mistakes have been made, but they are not comparable to those that would have occurred without such frozen sections. It is absolutely impossible to make diagnoses in 100 per cent of the cases. Those who have been in the habit of making

rapid sections for many years will be prepared to acknowledge that in the vast majority of cases the diagnosis can be made from the frozen section just as well as from the thinnest and most perfectly stained paraffin preparations. Wood reported that he had been using the frozen section in his hospital for twenty-five years and that he employed it more and more. His assistant, he said, spent every morning in the operating room deciding what operation should be done. In my own series of 250 biopsies, Wilson's frozen section method led to a correct microscopic diagnosis in 244 cases.

In my opinion, it is not a question which method should be used, the macroscopic, the frozen section or the paraffin method, but in accordance with the teaching of the old school of pathology, whether examination of any tissue should be regarded as complete when omitting any of these three procedures. If Warthin employs his overnight paraffin method as the only routine, his procedure may in certain cases be as incomplete as that of others when they use Wilson's frozen sections as the only means of diagnosing surgical specimens. Henke, in his *Guide to Tumor Diagnosis* (1906), pointed out that the microscopic diagnosis of fresh tissue is neglected without reason by those who rely only on the modern embedding and staining methods, and that in some cases only the examination of fresh tissue gives the possibility of recognizing the finer cell structures as they exist in the living stage. The method of making cell smears from the cut surface of unfixed tissue, which was recommended recently as something new by Dudgeon, is really the oldest technic used by diagnosticians of tissue.

The only disadvantage of the frozen method is that very small particles are wasted by this procedure, and the employment subsequently of other methods of diagnosis is prevented. There is however, one new rapid method, almost as reliable as that of Wilson, which is free from this disadvantage, namely, that developed by Terry. The entirely new principle of Terry's sections is that instead of having to cut tissue very thin to get histologic detail, relatively thin sections are made with a biconcave razor and stained only on one side superficially with a polychrome stain. The slice of moist tissue, with the stained side uppermost, is examined with artificial transmitted light. The advantages of this ingenious method are that it is extremely rapid, inexpensive and noiseless and can be used in the operating room without elaborate preparations. Few artefacts are encountered, since freezing, fixing, heating or dehydrating are completely avoided. Many of the cells are still alive when examined under the microscope. It is a truly supravital stain, and cells are studied as nearly as possible as they are in the living body. High, as well as low, powers of the microscope may be easily employed. If the technic is good, oil immersion examination of the tissue is possible. By substituting for the original Unna's

stain a neutralized polychrome methylene blue, the method is very satisfactory both on fresh and on formaldehyde-fixed tissue. Terry's sections are preferable, in my opinion, to Wilson's technic, especially when a rapid diagnosis is wanted on small fragments of tissue. The preliminary cutting of razor sections does not prevent the later use of paraffin embedding. Even thin razor sections are usually thick enough to be cut in celloidin or paraffin. Moreover, the staining of sections with polychrome methylene blue does not interfere with the subsequent staining of these with other stains, for the methylene blue is extracted completely when the tissues are run through alcohol. Therefore, the objections brought out against Terry's method, that the sections are not permanent, are without weight, because it should be the rule to follow the rapid sections in every case with permanent slides that can be kept on file as valuable records. For the preparation of these permanent slides, I prefer a careful embedding in paraffin, extending the processes of fixation and dehydration over several days, which—in my hands—gives much better results than Warthin's rapid overnight paraffin method.

Terry has never claimed that his method can be applied in every case. According to him, with his technic it may be hard to cut tissue consisting of calcium or bone and extremely soft or friable tissue. He found it difficult to stain necrotic tissue or tissue covered with mucus or colloid. Light cannot readily be transmitted through opaque or darkly pigmented, especially hemorrhagic, sections. The limited applicability may be overcome in many instances by short fixation of the specimen in hot formaldehyde. The consistency of soft particles will thus become more suitable for cutting, and tissue with large amounts of mucus are more easily stained. In my experience most malignant tumors involving bone have offered little difficulty, because they usually contain masses that are easily cut with the razor.

Terry tested his method on 7,000 malignant tumors, and in 98 per cent of the cases his microscopic diagnosis checked satisfactorily with that made by the pathologists at the Mayo Clinic from frozen sections. However, Terry himself does not suggest that the razor section method should replace other procedures; instead it should be employed in addition to other methods.

Christeller, a German master of the microscopic art, believed that Terry's method opens a new era in the field of biopsy. He regarded it as superior to other rapid methods, because in a very short time several different areas of a tumor can be cut and examined, permitting a continuous microscopic control of the surgical intervention. Fat tissue that cannot be cut by the freezing microtome is very suitable for Terry's method. Christeller was able to distinguish every finer detail of the nuclear and plasmatic structures of the cells; in 104 surgical specimens,

including 40 malignant neoplasms, only three times was it impossible for him to make a correct diagnosis, owing to the atypical structure of the tumors, which required embedding and special staining methods. In Christeller's opinion, the razor section method is unsurpassed in rapidity and accuracy and should be accepted as standard procedure in the operating room. It will bring the pathologist to the operating room and give him larger opportunities for improving his diagnostic abilities by the close cooperation with the surgeon, so indispensable in deciding on difficult cases of tumor.

In my recent series of 368 biopsies, Terry's method allowed the same histologic diagnosis as paraffin sections in 94 per cent. In 98.08 per cent both microscopic diagnoses were identical in regard to malignancy and benignancy. My results are therefore in accord with the view held by Wood that in the vast majority of cases a correct microscopic diagnosis can be made in the operating room in a few minutes after removal of the specimen, but that it is impossible to make correct diagnoses in 100 per cent of the cases. To do justice to the patient, no examination of tissue can be regarded as complete, if all three diagnostic methods—the inspection and palpation of the gross material, the microscopic examination of supravital preparations and finally the leisurely study of paraffin or celloidin sections—are not employed. The knowledge of tumors has run considerably ahead of the general training in pathology, so that, even with the use of all three methods, errors in diagnosis will still be too frequent.

#### CLINICAL VALUE OF BIOPSY

The opinions of clinicians and pathologists differ widely on the clinical value of biopsy. While Schmieden expressed a desire to limit the indications for exploratory excision as much as possible, and Krecke said that he regarded as the best physician the one who invokes the aid of the microscopic diagnosis only in exceptional cases, other surgeons—Kappis, Toelken, Loehr, Batzdorf, Coenen—stated that they require biopsies on all accessible tumors that cannot be diagnosed by other clinical methods. The more neoplastic diseases that Kappis saw, the more skeptical he became, he said, regarding the accuracy of the clinical preoperative diagnosis. Horder stated at the international cancer conference in 1928, that there is possessed in biopsy a diagnostic means approaching nearer to certainty than any other. Bloodgood expressed the belief that when people become more enlightened by educational campaigns, and the patients with tumors come into the hospital early after the first symptoms are noticed, the diagnosis will rest more and more with the pathologist.



Wood pointed to the fact that even today at least half of the malignant tumors that occur are so inaccessible that an early diagnosis can in no sense be made, and that even the accessible tumors are so rarely diagnosed in the early stages that only about 20 per cent of them are susceptible of operative treatment with a probability of cure. The education of the population has been extremely effective, and as a result surgeons and roentgenologists are asked to diagnose and treat tumors in a stage much earlier than that in which they were seen a few years ago. As a tumor that is easily diagnosed by the classic textbook symptoms is in most instances already beyond any possibility of permanent relief, and as those in which effective intervention may be expected to offer cure are often in the stage in which the clinical diagnosis cannot be made with certainty, Wood concluded that the pathologist is assuming a position of importance which he has not held since the diagnosis of tumor began. Most of the successful operations on cancer are exploratory in principle.

Reiman, Dietrich, Henke and Hanser emphasized the great importance of biopsy in doubtful tumors that would require a dangerous or mutilating operation for relief. More conservative is the standpoint of Maresch and Ewing. They stated that the resort to exploratory excision is a confession of ignorance. It is possible by long training to recognize the nature of most accessible tumors by various clinical signs, and the hasty resort to microscopic diagnosis tends to hamper the development of other diagnostic methods and of general clinical judgment. In not a few instances the clinical symptoms are more specific than the microscopic structure of a section of tissue. The microscope should be employed, therefore, only after other means have failed. There will, however, always, according to Ewing, remain a large number of conditions in which the fullest possible clinical analysis leaves doubt as to the nature of the disease, and when important variations in treatment depend on positive diagnosis, the microscopic evidence is essential. It is no longer possible to content oneself with the simple report that the growth is carcinoma or sarcoma. It is necessary to know exactly what type of carcinoma or sarcoma is present, what the extent of the disease may be, what degree of malignancy is concerned, and what the natural history of the disease will reveal. In other words Ewing urged the pathologist to form a clinical diagnosis and not rest merely on a histologic report. Only a few authors have tried by statistics to compare the relative accuracy of clinical and anatomic diagnosis of tumor. The most careful study of this question was undertaken by Fischer, comparing the two methods on 1,700 surgical specimens. The clinician's diagnosis was correct in 68 per cent of the cases; the pathologist's, in 91 per cent. In 14.5 per cent, the clinician had pronounced the growth malignant, while the pathologic examination revealed a benign condi-

tion. In 780 cases of tumor the clinical diagnosis was correct in 61 per cent; the pathologic microscopic study, in 91 per cent. In 18 per cent, the clinical diagnosis of malignancy could not be confirmed by the microscopic study of the surgical specimen.

MacCarty, reviewing the large surgical material of the Mayo Clinic, found that of 1,213 surgical cases, 16.4 per cent came to operation with a doubtful clinical diagnosis. Twelve per cent required biopsy, and in 17.5 per cent the histologic diagnosis, made from frozen sections during operation, changed the prognosis and the operative treatment. In 0.5 per cent of all surgical cases—including hernias and fractures—malignant tumors were discovered by the routine microscopic examination, the surgeons not having suspected it before or during surgical intervention.

In a series of 350 tumors that required biopsy I confirmed the clinical diagnosis by microscopic study in 232 (67 per cent); in 72 cases the clinical diagnosis was doubtful, and in 46 it was wrong. Twenty-five tumors regarded by the clinician as malignant proved to be benign, and in 21 cases of malignant neoplasms, as recognized by the histologic examination, the true nature of the process was not suspected previous to the exploration.

With the advent of radiation treatment, the microscopic examination was regarded by Regaud of still greater value than in the exclusively surgical era. The histologist was formerly useful but not absolutely necessary to the surgeon for confirming in advance of operation an uncertain clinical diagnosis. He has now, according to Regaud, become the indispensable collaborator of the radiotherapist. Analysis by biopsy not only has to facilitate the diagnosis of malignant tumors, but has to determine also their special variety. Radiotherapeutic technic is frequently influenced by a detailed knowledge of the histologic character of the tumor in a given case. In the Radium Institute at Paris, Regaud performs biopsy as a routine in every case, and the pathologic report is awaited before treatment with radiation is started. At the international conference on cancer in 1928, Marie emphasized that not only is the histologic examination necessary before the treatment with radiation is begun, in order to determine whether the patient is suffering from cancer and if so, of what type, but it must also be made in the course of treatment, for the purpose of determining whether the destruction of cancer cells is complete and to avoid confusing a necrosis due to excessive dosage with a recurrence due, on the contrary, to an insufficient dosage. Photomicrographic records of biopsy specimens made at different periods should accompany the clinical observation of the patient during treatment.

From the conflicting opinions of various authors it would seem that any general acceptance or condemnation of biopsy cannot be made.

Each organ offers a special problem, and the wisdom of resorting to probatory incisions must be determined for each particular case.

*Cervix and Uterus.*—It was for the diagnosis of gynecologic lesions that biopsy in its modern form was first devised, and it expanded in usefulness from there to other surgical fields. In no other specialty has the microscopic diagnosis ever played a more important rôle. Novak was convinced that biopsy of the cervix and diagnostic curettage are not resorted to as frequently as they should be. The factor of duration is more important in determining the patient's fate than are such factors as the method of treatment or the histologic classification of the tumor. The importance of biopsy lies, therefore, in the fact that on this procedure dependence must be placed for the recognition of the really early cases of uterine cancer. Even with the most expert pathologic study, in Novak's opinion, there will be a small residue of cases in which a positive diagnosis is difficult or impossible, but these cases constitute only a comparatively small proportion of those in which biopsy or diagnostic curettage is indicated. It is not conscientious to take a chance that a lesion is benign, nor, on the other hand, is it justified to assume that a lesion is precancerous and to do a radical operation. Elimination of cancer by biopsy means that a certain number of women will be saved from unnecessary and grave radical operations, and that others will be spared prolonged, expensive and harrowing radiotherapy.

Frank has seen, during a clinical experience of twenty years, but 2 cases of early cancer of the cervix in which histologic examination was really needed to confirm the diagnosis. In all other cases the clinical criteria were unmistakable. In the numerous cases in which doubtful cervical conditions were encountered, microscopic examination of the excised portion, showed them to be nonmalignant. For this reason Frank condemned the tendency to remove uteri on suspicion only. It has, according to him, led to a craze for hysterectomy comparable to the Batty craze for oophorectomy of the late seventies.

Meyer reported that in his experience, embracing 2,000 biopsies and diagnostic curettages every year, he had found exceptionally few cases in which the microscopic diagnosis remained doubtful and a biopsy had to be repeated. Extensive follow-up studies substantiate the confidence that he placed in the "Stueckchendiagnose." Of 43 patients whose conditions were diagnosed by the clinician as cervical cancer, but in whom microscopic examination of excised tissue had revealed benign lesions, all remained perfectly well after conservative treatment. In the same way, in 107 cases in which a certain diagnosis had been made by the histologic study of curettings, in not one did the subsequent course belie the histologic diagnosis. Hirschberg, in studying the large biopsy material of the Woman's Hospital at Leipzig, confirmed the clinical

diagnosis of cervical carcinoma in only one third of the cases, while in the rest microscopic examination revealed benign lesions. One case of syphilis and another of tuberculosis were diagnosed by the gynecologist as cancer of the cervix. In 235 of 244 diagnostic curettages, the clinical diagnosis was doubtful. There were only 22 malignant neoplasms, as evidenced by the histologic study. In 4 instances, the clinician made a diagnosis of cancer, which proved incorrect by the pathologic examination. Only in 5 curettages did the histologic diagnosis remain doubtful, on account of insufficient material.

In my series of 104 gynecologic biopsies, less than half of the cases were diagnosed correctly on clinical symptoms, in 34 the clinician's diagnosis was doubtful, and in 24 it was wrong. In 18 cases diagnosed clinically as cancer, biopsy revealed a benign lesion.

Stierlin reviewed 654 curettages and 213 excisions from the cervix, in cases in which malignancy was suspected. Of the 654 curettages, only 54 showed carcinoma; 1, sarcoma, and 1, chorionepithelioma. In 2 cases, the pathologist made, from the biopsy specimen, the diagnosis of carcinoma, but the removed uterus failed to show a neoplasm. In 1 case, a benign lesion was diagnosed after microscopic study of curettings, but sarcoma was found at operation. In another case, 4 curettings were made at short intervals and diagnosed as doubtful, malignant, benign and finally malignant. Stierlin arrived at the conclusion that it is not justifiable to perform hysterectomy on clinical suspicion only.

In Norris' 253 cases of cervical carcinoma, the clinical diagnosis was correct and positive in 81.4 per cent, wrong in 3.8 per cent and doubtful in 11.4 per cent. The high number of correct clinical diagnoses is explained by the fact that there were only very rarely early malignant lesions in Norris' material. In only 41 of the 253 cases was the disease confined to the cervix; in 45 it was inoperable; in 109 there was an involvement of the parametrium, and 17 cases were recurrences. In the same author's 101 cases of carcinoma of the fundus of the uterus, the clinical diagnosis was correct and positive in 57, doubtful in 24, and wrong in 20.

Dietrich examined 385 diagnostic curettages from patients with uterine hemorrhage. The microscopic study of the curetted material and the subsequent hysterectomy revealed carcinoma in only 11.2 per cent.

In 669 cases of cervical cancer, Pemberton and Smith had to rely on microscopic examination in 2.39 per cent, the clinical findings being inadequate. In 10 of the 16 cases of early carcinoma, biopsy was a life-saving measure. In the other 6 cases of early carcinoma, the nature of the condition was revealed by routine microscopic examination of trachelorrhaphy specimens. In their opinion, there should be no hesitation with regard to biopsy.

Whenever there is the slightest doubt as to the gross diagnosis, biopsy should be done as a preliminary before plastic work about the cervix is undertaken (Cooke). Novak mentioned several instances in which later examination of the excised tissue showed definite, though early, carcinoma. I remember such a case.

In a series of 1,808 cases of cervical cancer, Branscomb found 46 in which a malignant condition of the cervical stump was observed after supravaginal hysterectomy for a nonmalignant condition (33 myomas). Curettage previous to supracervical hysterectomy was therefore advised by Davis. The incidence of cancer associated with fibroid tumors of the uterus is according to various statistics above 2 per cent. Davis found 8 cases of cancer of the cervical stump following supracervical hysterectomy for fibromyoma.

The majority of gynecologists agree that in intra-uterine lesions the problem of early diagnosis can be solved only by diagnostic curettage and microscopic examination of the curettings. Not infrequently the appearance of the removed tissue to the naked eye is sufficient to establish the diagnosis with reasonable certainty. The combination of gross and immediate microscopic observation usually enables the surgeon to proceed at once with the radical operation, if cancer is found (Novak). While the value of biopsy in the early diagnosis of uterine cancer is generally recognized, the elimination of malignant growth by this method seems to be much less appreciated. Many gynecologists, Stoeckel, Pauchet and Stacy, consider panhysterectomy indicated without preliminary curettage in any postclimacteric metrorrhagia. The question arises whether the mortality of complete hysterectomy as a routine does not far exceed the prophylactic gain. In a recent paper, Benthin pointed to the fact that in a series of 131 cases of postmenstrual bleeding, cancer was the cause of metrorrhagia in only 56.

Senile endometritis in elderly women shows not infrequently slight ulceration, thus explaining the bleeding. At times polyps may produce similar metrorrhagia, though not nearly so often as in younger women. Hypertension as a cause of bleeding is observed in a considerable group of cases, and even ovarian tumor can be associated with postmenstrual uterine hemorrhage.

*Breast.*—There are some surgeons who are extremely confident about the accuracy of the clinical diagnosis of tumors of the breast. Beaver stated that the correct diagnosis of a lump in the breast can be made in more than nine tenths of the cases from the history and clinical examination. If an exploratory excision is necessary, the patient is told before the operation that the diagnosis cannot be determined with absolute certainty except by microscopic examination. Everything is prepared for a radical operation. In almost all of the doubtful cases—about 95 per cent—the character of the tumor can be determined from its

gross appearance after exploratory incision; in the rest, a frozen section is made and the operation continued according to the microscopic diagnosis. In Beaver's experience, the gross appearance is much more certain than a frozen section. According to Krecke, carcinoma of the breast can be recognized usually on clinical judgment. Of his 250 amputations of the breast, only 4 were found later by microscopic study of the removed breast to have been for benign conditions. Frozen sections must not be relied on, because the nature of the tumor can be determined more safely by the gross appearance during exploration. Klose and Sebening stated that in early cancer of the breast, histologic diagnosis is unreliable, and that therefore they amputate breasts with chronic cystic mastitis as a prophylactic measure, especially if the condition is associated with bleeding from the mamilla. Also Kueckens and Semb said that they do not trust frozen sections in making a differential diagnosis between chronic cystic mastitis and cancer.

Judd did not believe that every case of chronic cystic mastitis should be treated surgically, but that every solitary lump of the breast or any unusual nodule in association with a diffuse mastitis should be excised immediately for microscopic study. MacCarty, from the same clinic, held the view that every breast containing a tumor without clinical signs of cancer and every one showing a discharge from the nipple require wide excision of the mass or removal of the gland-bearing portion of the organ for immediate microscopic study. Only by this measure can the effects of incorrect clinical diagnosis and prognosis be prevented and improper surgical treatment—either too radical or too conservative—be avoided.

Wood mentioned that many women refuse a mastectomy, but accept the idea of an exploratory operation with frozen section diagnosis of the tumor. This conservative standpoint is perhaps helpful in stemming the present attitude of some surgeons that the breast of any woman over 35 years of age, if it contains a few nodules, should be promptly removed in toto.

Ewing stated that mammary diseases in which a probatory incision through sound skin is indicated are rare. When the question arises between chronic cystic mastitis and carcinoma, if any incision is made, it is usually the safest procedure to remove the whole breast and submit the entire organ for gross examination. If no malignant process is found, one has merely removed a menace to the patient, since any chronic cystic mastitis that has progressed so far as to suggest carcinoma frequently develops into carcinoma. In women under 35 years of age with localized chronic induration of the breast, it is perhaps permissible to excise a portion of tissue for frozen section. Ewing had known such a procedure to save the breast without subsequent recurrence of disease. In all such cases it is safer, however, to excise the entire sus-

pected area. If the excised tissue proves to be carcinoma, it can hardly be doubted that the best surgical principles have been violated, but it is perhaps too much to assert that the patient's chances have been jeopardized, if the probatory incision is immediately followed by radical operation. It is much more injudicious to remove a small portion of a diffusely indurated breast and base the subsequent procedure on the results of examination of a single piece of tissue. In chronic cystic mastitis, carcinomatous areas are often multiple and difficult to detect. The practice of aspirating cysts for diagnosis was reprehended by Ewing. In women under 30 years of age, a single cyst is usually unaccompanied by a malignant process, while after that age carcinoma is often found in the wall of the cyst or adjacent to it, or it develops later. At no age is the excision of a single cyst a satisfactory procedure. The variable circumstances under which tumors and chronic indurative diseases of the breast arise render it impossible to apply any rigid rules governing the probatory incision. Each case must be considered by itself (Ewing).

McGlannan did not confirm the contention of Bloodgood that blue-domed single cysts of the breast are always benign. In a series of 100 cases of cancer of the breast, he had 3 in which a carcinoma and a blue-domed cyst were associated. He therefore advised excision of all single cysts, together with a wide margin of mammary tissue, and examination of the surrounding tissue microscopically for malignant growth. I detected carcinoma in close proximity to a typical blue-domed cyst in 2 cases, which were regarded by the surgeon as benign at the exploration.

In Halsted's clinic, incomplete operations were done for malignant tumors in 1 per cent and radical amputations for benign lesions in 10 per cent (Bloodgood). Rarely was the frozen section employed during operation. Later as the percentage of cases with short duration of clinical symptoms increased, the complete operation performed for benign lesions increased from 10 to almost 25 per cent. According to Bloodgood's earlier records, 80 per cent of diseases of the breast, when first examined, were malignant, whereas recently only 17 per cent are malignant. In the past five years, immediate frozen section diagnosis of explored tumors of the breast has increased tremendously in Bloodgood's clinic, and in very early tumors malignant conditions are seen with a gross picture distinctly benign and benignant conditions with a gross appearance evidently malignant. When a mass in the breast of a woman over 25 years of age suggests chronic mastitis on palpation, exploration should not be delayed. After the twentieth year all palpable tumors in or near the breast should be removed. Discharge from the nipple, no matter what the character of such a discharge may be, is not a sign of cancer; the most frequent cause is a papilloma in a duct.

A papillomatous cyst should be widely excised, and the base carefully examined for invasive growth. Chronic abscess with cyst formation and chronic lactation mastitis may closely resemble cancer in gross appearance. Nothing but the frozen section will distinguish them (Bloodgood).

Frankenthal, Bloodgood, Klose and Sebening advised biopsy in eczema of the mamilla to exclude Paget's disease, especially in cases in which pigmentation or nonhealing fissures are present in the areola.

The value of biopsy in doubtful mammary lesions is evidenced by the statistics of Ladwig, based on his observations in Payr's surgical clinic. In 56 tumors of the breast exploratory incision was necessary in 24 instances, and in 17 the microscopic examination of frozen sections revealed carcinoma. My series of 90 cases of tumor of the breast showed a correct clinical diagnosis in 63 per cent; in 22 of the 46 cases of malignant tumor the clinical evidence was so definite that the surgeon performed the radical operation without previous exploration. In 28 of the 90 cases microscopic diagnosis was invoked during operation, and in 12 of these 28 cases, carcinoma was proved and radically excised under the same anesthetic. Of the 28 doubtful tumors, however, 16 were found to be benign on microscopic evidence, and the patients spared an unnecessary mutilating operation.

Fischer's study of the relative value of clinical and pathologic diagnosis in cases of tumor of the breast revealed that clinical diagnosis was correct in 71 per cent and pathologic diagnosis in 99 per cent.

From these statistics inferences may be drawn that clinical symptoms alone lead to a correct diagnosis in only two thirds of the cases of tumors of the breast. There cannot be any doubt that in the future clinical diagnosis will become more and more difficult, when the patients, stirred up by educational campaigns, seek examination earlier and earlier after the first symptoms are noticed.

*Bone.*—In his recent publications on tumors of bone, Bloodgood urged that biopsy should be a last resort. It is far safer for the patient to submit the x-ray film of the lesion to consultants than to perform a biopsy and submit the microscopic slides. It is also a mistake, in Bloodgood's opinion, to explore a lesion of bone without knowing the result of the Wassermann test. Syphilis of bone is perhaps the most protean of all osseous lesions and may simulate osteomyelitis, a benign tumor or a sarcoma. It is not unusual for a lesion that presents in the x-ray picture the features of a typical sarcoma to prove to be syphilitic. Geschickter reported 2 cases of syphilis of bone, in which the microscopic examination at exploration—before the result of the Wassermann test was known—failed to reveal the true character of the lesion.

Biopsy should not be done, according to Bloodgood, when a periosteal or diffuse tumor is situated on an upper extremity or above the middle third of the femur. In these locations, radiation is all that can be



offered for the malignant lesions, and will not be harmful if the tumor is benign. Biopsy may be omitted when a tumor of bone is clinically inoperable, and when x-ray therapy is used palliatively. It also may be omitted in a tumor that is malignant in the roentgenogram and that shrinks rapidly under irradiation. Furthermore, in experienced hands exploration need not be resorted to when a resection of the bone is performed for a tumor diagnosed malignant on roentgen examination.

Since Bloodgood believed that in situations below the upper third of the femur amputation offers more for a permanent cure of sarcoma than radiation, he favored biopsy before amputation of the leg. If the clinical examination cannot absolutely rule out sarcoma, and the tumor—periosteal or diffuse—is so located that it can be amputated or resected, there should be no delay in performing the operation. The object of this operation is to explore, excise a piece for frozen section and if the diagnosis of sarcoma is established, to perform resection or amputation immediately. Sclerosing and osteoporotic sarcoma in the late stage gives a typical x-ray picture and should be diagnosed with rare exceptions without biopsy, but when there is only slight destruction of cortical or cancellous bone, it is difficult to differentiate sarcoma from diffuse osteomyelitis. In some of these benign bone-forming periosteal lesions, it requires great experience to recognize the condition, even with the microscope. In sclerosing osteomyelitis there is neither pus nor a sequestrum to rely on for a diagnosis, and also the histologic picture is deceiving.

With central tumors of bone, Bloodgood's attitude is different. The predominant central lesions are osteitis fibrosa and the giant cell tumor. Next in order of frequency comes the metastatic carcinoma. Chondroma, myxoma and sarcoma are infrequent. The multiple myeloma without evidence of involvement of other bones is very rare. The central sarcoma should be easily distinguished from the giant cell tumor and osteitis fibrosa by its fresh appearance and by the frozen section. The very rare aneurysm of bone can be differentiated, according to Bloodgood, from the giant cell tumor in the frozen section.

In central lesions of bone with intact bone shell, delay in operative exploration under x-ray therapy adds no risk. But there must be an exploratory operation to determine the nature of the process in a central tumor in case of nonhealing fracture or perforation of the bone shell. According to Bloodgood, biopsy is justifiable only when the patient has consented to resection or amputation, which are the operations of choice in the periosteal and diffuse sarcoma and may be the operations of necessity in central lesions like chondroma, myxoma, myxochondrosarcoma, metastatic tumor and even in some instances of myeloma. If there is any doubt from the x-ray picture or from the microscopic study of the lesion as to its definite malignancy, it is safer to treat the condition as

innocent, because the element of error is largely in favor of the benign condition and the probability of curing a malignant tumor is as yet far too small to justify amputation or resection, unless the diagnosis of cancer is as certain as it is possible to make it. In cases of central sarcoma there are no five year cures to date. All the cures concern periosteal and diffuse sarcomas involving bone of the lower extremity below the upper third of the femur (Bloodgood).

In his treatise on surgical pathology of the bones (1931), Hertzler emphasized that the pathology of malignant conditions of bone would be much elucidated if exploratory incisions and microscopic study were more frequently resorted to. The earlier the exploration the more needful in microscopic examination of the tissue removed. Formerly, unless amputation was to follow the demonstration of a malignant condition, exploration was not considered justified. Now knowledge so gained may determine whether amputation or radiation should be the treatment of choice. The chief purpose of exploration is, however, in Hertzler's opinion, to avoid amputation in case of a nonmalignant lesion. Exploration to determine the type of malignant growth is justified only in the hands of those whose experience enables them to know what to do with the knowledge thus gained.

Hertzler pointed to the limitation of microscopic diagnosis in lesions of bone. In differentiating inflammation and neoplasm, an opinion should never be given on the microscopic observations alone, since the cytologic changes in the soft parts in osteomyelitis may closely resemble neoplastic processes. In chondroma it is unsafe to make a diagnosis on histologic evidence alone. Invasion of blood vessels may occur in tumors without cellular evidence of malignancy. In chondrosarcoma, according to Hertzler, it is impossible to say from a given slide that it represents a malignant tumor of cartilage. Kolodny said that in the large majority of cases of osteogenic sarcoma the experienced diagnostician arrives at a definite diagnosis from a careful analysis of the clinical and roentgenologic data and the result of the radiation test. There are, however, exceptional cases in which one is unable to arrive at a definite diagnosis. In these cases an exploratory incision is unavoidable. The frequently encountered regressive changes, spontaneous or after irradiation, inflammatory changes, traumatic or infectious, and various peculiarities of the histology of osteogenic sarcoma make it desirable to diagnose a malignant skeletal tumor from the gross appearance without dependence on the microscopic picture. Sometimes even a diagnosis based on examination of numerous slides may not be correct. If, in osteogenic sarcoma, tissue obtained through biopsy is not representative enough to allow a correct diagnosis, even more is this the case with Ewing's sarcoma. There are numerous examples of the pathologist supporting the erroneous diagnosis of osteomyelitis made clinically and roentgeno-

logically in the presence of Ewing's sarcoma. The diagnostic limitations of pathology combined with the dangers of exploratory incision in Ewing's sarcoma require that a therapeutic radiation test should be substituted for exploration in suspected cases of Ewing's sarcoma. In myeloma, exploratory incision is both undesirable and unnecessary because of the promptness with which myeloma responds to irradiation. The diagnosis is established early from the roentgenogram.

In giant cell tumor the therapeutic test is of less importance. The difficulties encountered in histologic diagnosis of a doubtful giant cell tumor are great and to those little initiated in the pathology of tumors of bone insurmountable. As a general rule, when the clinical findings and the roentgenogram are baffling to the clinician, the histology is distressing to the pathologist. It may be safely argued that to those experienced in the pathology of tumors of bone the gross anatomy of a giant cell tumor frequently means more than the microscopic; and diagnosis from the gross specimen is more apt to be accurate than that from the slides. In the variants of giant cell tumor the histologic appearances are not infrequently misleading. For instance, in the myxomatous variation of giant cell tumor the histologic observations may suggest cancer, while the clinical and the roentgenologic features clearly indicate the benign nature of the lesion. The advanced cicatrizing stage of giant cell tumor may suggest fibrosarcoma, if other data are disregarded. The histologic observations are especially deceiving when the tissues have been taken from tumor masses fungating through a former incision made for biopsy. It is extremely hazardous to base a diagnosis on findings in these granulation tufts; the numerous mitoses here are mostly in the endothelial leukocytes (Kolodny).

The difficulty and often the impossibility of correctly interpreting the great variety of structures occurring in tumors of bone and in processes simulating them had steadily diminished Ewing's estimate of the value of biopsy. He stated that unless the structure observed is entirely typical, the pathologist is apt to mislead rather than aid the surgeon. When the histologic diagnosis is easy, the roentgenologic and other signs are generally equally clear. Ewing was therefore inclined to restrict biopsies to doubtful cases just before amputation. He regarded the therapeutic radiation test as more helpful to differentiation between benign and malignant medullary tumors. Under irradiation, the giant cell tumors slowly regress, and the bone shaft is restored, while the malignant osteogenic sarcomas, with rare exceptions, show no such response. One has, however, to consider that, as Holfelder stated, the initial reaction to treatment with the x-rays in sarcoma may give the impression that the growth has increased, and there is always considerable delay before shrinkage becomes evident. Many sarcomas of bone begin to regress in four weeks, but in others the improvement

appears only after from eight to ten months. Biopsy is employed by Holfelder in all tumors of bone from three to four weeks after the first treatment with x-rays. Also Pels-Leusden, Oehlecker and Hueck demanded biopsy in every tumor of bone that is to be subjected to radiation therapy, because without histologic evidence they would deny any claim of curing a malignant tumor.

The opinions of European surgeons in regard to biopsy in tumors of bone are not uniform. Putti rejected this diagnostic method because the pathologist is compelled to decide on the nature of a lesion that may differ widely in various areas from a fragment revealing only a limited aspect. Also Axhausen found that the value of biopsy in tumors of bone is very limited. Mueller and Schoene, on the other hand, believed that microscopic study of sufficient tissue, removed from the right place, often gives invaluable information. Konjetzny stated that he requires biopsy in all doubtful tumors of bone and especially in suspected giant cell tumor, to avoid unnecessary mutilating operations. Lubarsch was of the opinion that in numerous lesions of bone the experienced pathologist is able to diagnose from microscopic slides not only whether the disease is benign or malignant but, if it is malignant, the grade of malignancy. In general, he had found that it is impossible to determine the character of a tumor of bone only from microscopic study of excised tissue, and that in borderline cases the pathologic examination permits only a very guarded opinion.

Volkman regarded the histologic differential diagnosis between osteitis fibrosa and sarcoma of bone of definite value only when it confirms the clinical diagnosis. If the diagnoses disagree, the doubtful lesion should be treated conservatively. Two of his own cases were diagnosed by an experienced pathologist as central sarcomas, but the clinical course confirmed the clinical diagnosis of osteitis fibrosa.

*Skin.*—The clinician entertains a feeling of outrage if microscopic study does not furnish him the desired diagnosis in lesions of the skin, or if the observations are not in accord with his expectations. To give an appreciation of the situation, Highman called to mind that there are more than 500 so-called skin diseases of which the inflammations are usually not distinctive as to microscopic appearance. Tumors of the skin are distinctive enough histologically. In fact the clinical identification of neoplasms falls so far short of the microscopic evidence that the latter becomes an imperative aid in the diagnosis of tumors of the skin and in the recognition of the subtler types of nevi. All the connective tissue growths and most of the epithelial neoplasms, both benign and malignant, including such lesions as molluscum contagiosum, pointed condylomas and the like, possess characteristic minute structures. In infectious inflammations of the skin—provided the specific

parasite cannot be demonstrated—and in some other types of lesions, fundamental difficulties arise that render the microscope scarcely serviceable in diagnosis. Cutaneous syphilis, lepra, rhinoscleroma and tuberculosis in its multifarious aspects are often distinguishable from one another and from similar lesions caused by various fungi. But the seasoned microscopist would not have the hardihood to be arbitrary in borderline cases.

The so-called lymphodermias—that is, infiltration of the skin in leukemia, Hodgkin's disease and mycosis fungoides—are often typical enough to be identified by a shrewd observer; more frequently, however, this is not so nor can the prodromal phases of these conditions be recognized with any certainty.

Thus, according to Highman, histologic study of lesions of the skin is a first rate diagnostic aid only in the case of neoplasms. It is a fair diagnostic guide in granulomatous infections and in lymphatic infiltrations. Among inflammations, only lichen planus and urticaria pigmentosa present a distinctive microscopic appearance, but it is of no practical importance since these conditions are clinically obvious. In all other inflammatory conditions, although the microscopic structure is often suggestive, no justification exists for microscopic diagnosis. Therefore cutaneous histology aside from its bearing on neoplasms is of no great value in clinical diagnosis.

The dermatologic clinic of Riehl at Vienna uses biopsy in all doubtful lesions of the skin, and, if necessary, repeated diagnostic excisions (Arzt). Ascher regarded the biopsy in all cutaneous tumors as of the greatest diagnostic value. He remembered several instances in which the diagnostic excision of cutaneous nodules brought evidence of a primary tumor in internal organs (Kuettner, Krecke). Uhlenbruck and Gilardone stated that subcutaneous cancer nodules in the abdominal wall are usually lymphogenic metastases of abdominal tumors. Hematogenic isolated metastases in the skin of the thorax, abdomen or back are frequently the first signs of disseminated carcinomatosis. Without microscopic examination of these easily accessible tumors an entirely erroneous opinion will be held in some cases.

Klapp, Bange and Ernst stated that from one seventh to one tenth of all carcinomas originate in the skin of the face. Often they appear for many years as insignificant lesions, and for early diagnosis, biopsy with microscopic study of the tissue is essential.

Volkman urged histologic examination of every excised tumor of the skin to avoid prognostic mistakes. He reported the case of a melanoma that was removed as a clinically harmless nodule, but that later developed metastases. Bloodgood stated that biopsy is not necessary in small tumors of the skin provided they are totally removed with a sufficient margin, should cancer be present. It is a dangerous

and much abused procedure to shell out small subcutaneous, subfascial and intramuscular tumors, because in the case of early malignant growth the clinical diagnosis is often unreliable. Bloodgood found that 33 per cent of the cutaneous sarcomas under his observation were recurrences of lesions that at the time of the first operation had been small and apparently innocent. In all these cases the tumor had been enucleated. When the tumor is so large or so situated that the radical removal on the diagnosis of possible malignancy would mean mutilation or would endanger a great nerve or blood vessel, one must expose a small area of the tumor for biopsy. Cautery and immediate diagnosis from frozen section are regarded by Bloodgood as the methods of choice.

In Delbanco's experience, histologic examination of multiple epitheliomas of the skin seldom threw light on the question of their benign or malignant character. Freudenthal also stated that the histologic features of multiple epitheliomas and senile warts can rarely be relied on, since clinical signs of malignancy may occur without any apparent histologic change.

*Mouth.*—That clinical diagnosis of intra-oral lesions is not as reliable as Birkett would believe is evidenced by the lamentable fact that many tuberculous and syphilitic tongues adorn the shelves of pathologic laboratories (Wood). Most of the clinicians agree that every suspicious lesion, especially in men over 40 years of age, should be carefully examined, and this, according to Heidler, Baumecker, Quick, Bruening and Mowat, includes biopsy. When the lesion of the mucosa of the mouth is so small that it can be removed without mutilation, its radical excision without previous biopsy would be advised by Krecke and Bloodgood. It is difficult to distinguish clinically and microscopically the benign from the malignant ulcer of the gum. When the ulcer of the gum is about a tooth and the radical removal of this ulcer would mean extraction of the tooth, biopsy is indicated. Pfahler pointed out that all patients with doubtful lesions of the buccal cavity should have both a microscopic and a serologic diagnosis, for a large percentage of cancers of the mouth develop on old syphilitic lesions. Therefore a positive Wassermann reaction does not eliminate the diagnosis of cancer and should not delay the treatment. Likewise a microscopic diagnosis of cancer does not eliminate the possibility of associated syphilis.

*Esophagus.*—As early as 1901, Gottstein called attention to the great diagnostic aid of biopsy in cancer of the esophagus, when he reported several cases in which this method was of decided value. According to Jackson, the only certain way of making a diagnosis of esophageal cancer early enough to be of any avail is by esophagoscopy

and removal of tissue. All other methods are late at best and often erroneous at worst. To warrant a transthoracic esophagotomy on a man in good general condition necessary to survive the major operation, an absolutely positive diagnosis is required, and this only the histologist can give (Jackson).

*Larynx.*—Tucker pointed out that in intrinsic cancer of the larynx, early diagnosis offers a lasting cure in from 70 to 80 per cent. This is a much higher percentage of cures than can be obtained in cancer in any other location in the body. It is therefore imperative that every physician should recognize the symptoms, appearance and means available for the accurate diagnosis of incipient carcinoma of the larynx. In every case of early laryngeal cancer, the conclusive diagnostic step should be biopsy. If the result is negative, repeated specimens should be taken at proper intervals and under proper aseptic precautions until conclusive evidence is obtained. In early cancer the appearances in the mirror are not characteristic. Sorensen, the German authority on laryngeal carcinoma, reported that he never performs a radical laryngeal operation before the suspicious tumor is proved to be a carcinoma by microscopic study. There are limitations in the histologic method, as evidenced by the report of several cases in which the microscopic diagnosis was belied by the clinical course.

*Bronchus.*—Primary bronchial carcinoma constitutes, in Jackson's opinion, a mild, slowly metastasizing, relatively benign disease. Only early diagnoses are required to enable the surgeon to obtain a good percentage of cures. The only way to make the diagnosis early is by bronchoscopy and histologic confirmation.

*Lung.*—It is a deplorable fact that the patient with cancer of the lung is usually treated for a few years under an erroneous clinical diagnosis. Junghanns, for instance, found that in a large proportion of cases of carcinoma of the lung verified at autopsy the diagnosis on clinical grounds was not made. The condition was frequently confused with tuberculosis. In carcinoma of the lung, biopsy of a secondarily involved lymph node enables the pathologist to determine the presence of malignant growth and also its general type. With the help of careful physical examination and roentgenoscopy, the diagnosis of carcinoma of the lung may thus be established. On rare occasions the pathologist may make a diagnosis of cancer of the lung from fragments of the tumor discovered in the sputum. Betschaert reported the diagnosis of pulmonary cancer by this method in 1895 and reviewed the scant literature on the subject, which revealed 3 previous cases. Hellendahl reached a diagnosis of sarcoma in 2 instances by a histologic examination of a specimen of tissue obtained by diagnostic puncture of the lung. He quoted Kroenig as the only one preceding him

in the use of this method for diagnosing carcinoma of the lung. The examination of the pleural fluid for neoplastic cells has been investigated particularly by Specof, who was able to make a diagnosis in agreement with the subsequent observation at autopsy in 79 per cent of 38 pleural fluids. Thus it may be seen that the pathologist can have an important part in establishing the diagnosis of carcinoma of the lung during the life of the patient.

Sharp recommended diagnostic aspiration in doubtful tumors of the lung, should the lesion be in one of the upper lobes or in the parenchyma near the periphery of the lower lobes, where bronchoscopy is impossible. The basis for his judgment of the advantages of this diagnostic procedure was a single case of his own and 2 cases reported by Martin and Ellis.

*Stomach.*—Scott stated that it is quite impossible to differentiate clinically between benign calloused ulcer of the stomach and carcinoma, even at operation. Sometimes the macroscopic and even the histologic examination of the resected ulcer do not settle the question. In the base or the edge of an ulcer, carcinoma cells may be very abundant or may be found only with difficulty. Thomson, Graham and Thalhimer and Wilensky each reported one case in which many microscopic sections failed to demonstrate carcinoma, but in which the latter was found in regional lymph glands. The histologic examination of resected calloused ulcers which were diagnosed by the clinician as benign revealed carcinoma in the following percentages: Moynihan, 18 per cent; Payr, 26 per cent; Kuettner, 30 per cent, and Finsterer 21 per cent. Aschoff, who is extensively quoted as an opponent of the frequency of carcinoma ex ulcere, expressed surprise at the large number of resected specimens resembling macroscopically the ordinary round ulcer, but proving histologically to be carcinoma. From the statistics of different clinics it would seem to be a conservative estimate that in from 10 to 20 per cent of chronic gastric ulcers carcinoma is present, whether it has arisen from a chronic ulcer or has been a primary carcinoma with secondary ulceration.

Another line of evidence corroborates this significant proportion of indurated ulcers that appear benign but prove to be malignant. In 334 cases in which gastro-enterostomies were performed for ulcer of the stomach and duodenum, von Eiselberg found that 13 of 41 remote deaths were known to have been due to carcinoma of the stomach, a probable incidence of carcinoma in gastric ulcers of from 15 to 20 per cent. The difficulty of diagnosing carcinomatous gastric ulcer even from microscopic slides is elucidated by the observations of Balfour. In 1,280 patients with clinically benign ulcer of the stomach, followed for an average of three and six-tenths years after operation there were 195 deaths, at least 75 of which were determined as due to carcinoma;



many more on which the data were inconclusive probably were due to this cause. On a careful reexamination of surgical specimens classified as ulcer at operation in cases in which the patients subsequently died of gastric carcinoma, it was found that half of them showed pathologic evidence of malignancy that had been overlooked. On account of this great difficulty of the differential diagnosis between benign and malignant ulcer, Gulecke and other surgeons would perform resection of the stomach in every case of calloused ulcer, if technically possible. And MacCarty would have every excised gastric ulcer, regardless of the clinical and the gross pathologic diagnosis, studied during operation for the presence of early carcinoma, in order to bring about resection instead of excision, when possible, and in order to give a correct prognosis.

Gastroscopy has been of less aid in early diagnosis of gastric carcinoma than the roentgenologic and general clinical examination (Gutzeit). Malignant transformation of a chronic ulcer cannot be determined with the gastroscope. The excision of tissue from a suspicious ulcer through the gastroscope, which has not yet been possible, may be of diagnostic aid in the future.

*Intraperitoneal Tissues.*—Bloodgood emphasized the great value of biopsy in intraperitoneal tumors. Multiple tuberculous nodules throughout the peritoneal lining may resemble carcinoma and the reverse. The sulphur granules of pancreatic necrosis may contain metastases from a pancreatic carcinoma. The removal of a lymph gland near a tumor of the stomach or of the intestines may show metastatic growth, while the absence does not exclude malignancy of the doubtful tumor.

*Rectum.*—In biopsies on tumors of the rectum, careful selection of the right portion is essential (Mandel), since there are several cases on record in which the excised tissue did not reveal any malignant growth, but in which the subsequent clinical course proved the presence of carcinoma. Most of the rectal cancers may be diagnosed on clinical grounds. Only the tumors without ulceration require a more frequent use of well and deeply performed diagnostic excision. Hochenegg, with his wide experience in this special type of tumors, observed 4 cases in which, although he had diagnosed clinically inoperable rectal carcinoma, the patients—after simple colostomy—made an unexpected recovery.

Reichle and Tietze stated that the diagnostic value of biopsy in rectal tumors is high, provided the microscopist is familiar with the special histologic conditions of this region.

*Urinary Tract.*—All papillomas of the urinary tract, according to Lubarsch, represent quiescent stages of malignant tumors, a view accepted more and more by surgeons and pathologists (Macalpine,

Isaac, Wehner). Broders included in his group 1 of carcinomas of the bladder tumors that most pathologists call benign papillomas; according to Wehner, there are insensible gradations from benign to malignant stages of papillomas of the bladder. Only the most careful histologic examination of the whole tumor and pedicle reveals, as signs of malignant tendencies, atypical proliferation of cells, larger size of nuclei and nucleoli, asymmetric mitoses and infiltration of the stroma by round cells. (Zuckermandl, Lichtenstern, von Fritsch).

The following factors seem responsible for vesical neoplasms being separable from tumors elsewhere in the body and not amenable to the usual classification into benign and malignant tumors: (1) so-called benign papillomas tend to implant themselves in other parts of the bladder and in the suprapubic wound after open operation; (2) recurrences after destruction of a benign papilloma of the bladder are common; (3) frank carcinoma may develop after the cure of a papilloma that was found benign by microscopic study (Aschner, Macalpine, Frontz).

Although many urologists admit, as Geraghty did, their inability by cystoscopic inspection to differentiate benign and malignant papillary growths, they nevertheless choose to dispense with the biopsy. Macalpine depends for his diagnosis entirely on the reaction of the cystic tumor to diathermic treatment. A tumor that disappears under this treatment is, in Macalpine's opinion, probably benign, as diathermy seems to aggravate the malignant tumor. Frontz pointed out that the histologically benign and malignant papillomas usually respond equally well to the same type of therapy, radium combined with fulguration; therefore, a differentiation between benign and malignant papillomas was rejected by him. In selecting the best form of therapy he relied, he said, entirely on the data obtained from cystoscopy and palpation.

Aschner, on the other hand, classified tumors of the bladder, in harmony with the terminology of tumors in general and with clinical requirements, as benign and malignant. Basing his view on 242 biopsies of tumors of the bladder, he arrived at the conclusion that the microscopic diagnosis is potentially reliable in 97.5 per cent of cases. Microscopic evidence of infiltration was found in 78 per cent of papillary carcinomas, whereas clinical signs of infiltration were observed in only 50 per cent. Aschner regarded it as of the utmost importance that biopsy should be made before resorting to treatment for suspected malignancy, because inflammatory lesions may closely simulate cancer. Failure to do this has led to harmful radical surgical interventions (Aschner, Joelson and Lower).

Limitations of the histologic method are encountered in two types of carcinoma of the bladder. In rare cases of infiltrating papillary

carcinoma the cell growth is indistinguishable histologically from that of benign papilloma, yet the tumor invades the pedicle and the wall. In these exceptional cases the results of biopsy are negative, unless tissue has been removed from the infiltrated base. And in some cases of diffuse papillomatosis it is evident that only a few of the numerous tumors are examined microscopically, so that a malignant one may readily be missed.

*Prostate.*—The prostate gland according to Ewing is not accessible to probatory incisions, and a negative report on a portion of tissue seems to him of little value. This skeptical view was substantiated by Hirsch and Schmidt, who detected very small malignant growths in prostate glands removed with the clinical diagnosis of benign enlargement. Many areas of the prostate gland have to be examined carefully with the microscope for malignant changes; otherwise these small malignant foci escape notice. Young mentioned the fact that about 20 per cent of elderly men requiring treatment for obstructive conditions of the prostate are suffering from carcinoma. If the diagnosis is doubtful after roentgenologic examination, exposure through the perineum, with biopsy, is desirable.

Ferguson recommended biopsy by aspiration of tissue with a Record syringe as a routine procedure for neoplasms of the prostate. In his hands this method has given more satisfactory results than any heretofore proposed. In my opinion, the only reliable method of biopsy in an enlargement of the prostate is the gross and microscopic examination of the whole prostate immediately after removal, so that in case of a cancer the operation can be extended. In my cases, Terry's method, with its possibility of examining in a short time many different areas, was superior to any other histologic method. Laewen called attention to the fact that only about 10 per cent of patients with carcinoma of the prostate survive the radical operation longer than three years. Since early cancer of the prostate cannot be diagnosed clinically, there is only one certain way of making a definite diagnosis, namely by the microscopic study of every prostate during operation, even if there is no clinical suspicion of a malignant condition. There are exceptional cases in which even this method fails. Hunt, for instance, reported a case of a definitely encapsulated adenomatous hypertrophy of the prostate gland which was readily enucleated and in which careful microscopic study failed to show any evidence of a malignant condition. The patient died, and microscopic examination of tissue removed from the prostatic capsule at autopsy disclosed carcinoma.

*Lymph Glands.*—That clinical diagnosis of lesions of the lymph glands is extremely defective is evidenced by the study of Fischer.

Only in 41 per cent of 108 cases was a correct diagnosis made by the clinician. In 21 of 45 cases diagnosed on clinical grounds as Hodgkin's disease, biopsy did not confirm the clinical diagnosis. In the early stage of Hodgkin's disease, also, microscopic diagnosis is not always possible, since the characteristic tissue changes may be absent for some time. In 15 of Barron's 24 cases, the diagnosis of Hodgkin's disease was definitely established after the first biopsy. One case was first diagnosed by the pathologist as probably tuberculosis; in another case, Hodgkin's disease and tuberculosis were ruled out on microscopic observations, but a second biopsy showed the changes of Hodgkin's disease.

The degree of accuracy with which the clinical examination and the pathologic report agree in regard to the involvement of the axillary glands in cancer of the breast was studied by Greenough. The practical importance of diagnosing axillary metastases is obvious because cure after amputation of the breast depends more on the absence of axillary metastases than on any other factor. In 215 cases, the lymph glands were recorded by the clinician as enlarged, and in 90 per cent of these cases they proved to be cancerous on histologic examination. In 135 cases, the axillary glands were diagnosed clinically as not enlarged, but in 40 per cent of these cases the pathologic examination showed the presence of cancer in the glands after operation.

Elkin, in his study of 41 cases of primary neoplasms of the lymph nodes, stated that, except in leukemia, biopsy and microscopic study is essential in the diagnosis of all these tumors. Also the unexpected finding of metastatic growths in lymph glands has furnished most valuable information of primary tumors in internal organs, completely unnoticed previous to biopsy. I recall cases of my own in which the histologic examination of an apparently primary tumor of the neck led the physician to the correct diagnosis of hypernephroma, colloid carcinoma of the stomach and carcinoma of the larynx and lung.

#### FUTURE OF BIOPSY

The scientific world awaits impatiently the future discovery of more efficient diagnostic methods, of which there is certainly need, at least for tumors not accessible to diagnostic incision. But it can be safely asserted that with the present microscopic methods, suffering and mortality from cancer could be effectively reduced, if the benefits of expert biopsy were available to every patient with cancer.

From statements made by leading cancer specialists all over the world it seems that the practice of biopsy is inadequate in many places, and that there is much need for more highly organized application of

the knowledge that is available. When Ewing, Greenough and Gerster made a survey of the medical service available for patients with cancer in the United States, they found that defects in the field of pathologic diagnosis form one of the most serious obstacles to the successful treatment of the patient with cancer, and they recommended that organized efforts be made to provide at least the minimum adequate service of this kind wherever diagnosis and treatment for cancer are offered to the public. At present it is a common practice of surgeons to send portions of tissue, not always skilfully selected, to the laboratories of general hospitals for diagnosis by pathologists who often have very inadequate experience in the diagnosis of tumors. This practice results in many inaccurate and erroneous diagnoses. Without criticizing the general competence of the pathologists who do this work, it should be recognized that the accurate diagnosis of tumors requires wide experience, and that persons of such experience are not always readily accessible. For this reason, free diagnosis of tumors has sometimes been provided in laboratories directed by experienced men, as at the Buffalo State Institute and the Huntington Hospital at Boston. Ewing and his co-workers recommended that this pathologic work be gradually concentrated in university, hospital or state laboratories which are known to be provided with men of considerable experience in this field of tissue diagnosis.

Wood stated that there are today few places where the pathologist can obtain a broad fundamental training in the diagnosis of tumors. Even the man who is thoroughly trained has no assurance that he will escape from the treadmill of routine laboratory work. Until it is recognized that the pathologist knows more about disease than most of the clinicians who outrank him in his hospital, and until advancement to the clinical staff is only through the laboratory, as it is in Germany, there will still be too few pathologists. Patients with cancer form only about from 3 to 4 per cent of the admissions to a general hospital. Therefore, it is absolutely necessary to concentrate a large number of cases of cancer in certain institutions in order that research and education may go hand in hand with the best therapy. Some small hospitals employ technicians to make their tissue diagnoses, with lamentable results.

Phemister pointed out that to make a diagnosis from frozen sections during operation is much more difficult than to do so later from sections of fixed tissue, and that there is needed the services of a person who has had considerable and more or less continuous experience with frozen section methods. For this reason, the work in the department of surgical pathology in hospitals in which teaching or research is not carried on should be done by specialists in surgical pathology rather

than by surgeons who devote part of their time to pathology. In the University of Chicago the section of surgical specimens is done by a member of the department of surgery who serves as surgical pathologist, and the work is checked by the department of general pathology.

According to Bloodgood, the diagnosis of pathologic lesions through the microscope is largely a matter of memory, and this requires special training and continuous operation. Every student of surgical pathology should examine at least a dozen sections daily. In the diagnosis of cancer in its earliest stages there will be a great demand for pathologists trained in microscopic diagnosis from frozen sections. It is often possible to combine pathologist and operator in one individual. This seems economically the best ultimate solution; it makes for a greater career for the pathologist to be an operator, and it makes the operator a far more useful person if he is a pathologist.

The organization favored by the German surgical clinics, whereby members of the clinical staff serve as diagnosticians of surgical specimens, is regarded as far superior to that of the American general hospital by Wood, Bloodgood, Novak and others. There cannot be any doubt that in the United States the clinical pathologist of the average hospital who not only is responsible for the pathologico-anatomic diagnoses, but has to supervise also the chemical, serologic, bacteriologic and metabolic work and sometimes directs even the x-ray department, can devote but a small part of his time to the diagnosis of tissues. But the German system must not work so flawlessly either, since Meyer criticized it severely in a recent address:

The histologic diagnosis of cancer lacks at present the opportunity of a fundamental training. Most surgical pathologists have to acquire their knowledge by their own efforts, learning from experience and especially from their own errors. There is a striking need for professional experts practicing and teaching the pathology of tumors. The difficult task of diagnosing biopsy material, deciding on life and death of a patient, should not be entrusted to a clinical assistant—often the youngest—as part time work. The large surgical clinics should have full-time directors of their laboratories who, without being handicapped by financial worries, could attain mastership in this difficult art by a lifetime of highly specialized work.

In the attempt to improve the present situation, the first and most important step must be, in my opinion, to take measures to attract to the field of surgical pathology young physicians of the highest caliber. A career in tumor pathology must be made as interesting and satisfactory from every point of view as a career in any other phase of medicine. Surgical pathology—called by R. Meyer the stepchild in the family of medical specialities—will then perhaps grow up to a brighter future.

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## Notes and News

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**University News, Promotions, Resignations, Appointments.**—William T. Belk has been appointed consulting pathologist at the Monmouth Memorial Hospital, Long Branch, N. J.

George P. Berry, at the Rockefeller Institute for Medical Research, has been appointed professor of bacteriology and assistant professor of medicine in the medical school of the University of Rochester, Rochester, N. Y.

Leonard G. Rowntree, director of clinical investigation, the Mayo Clinic, Rochester, Minn., and a professor of medicine in the University of Minnesota, has resigned to accept the directorship of the newly created Philadelphia Institute for Medical Research.

Frank A. Hartman, professor of physiology in the school of medicine of the University of Buffalo, has been awarded the Chancellor's Medal for his work in extracting the hormone (cortin) of the adrenal cortex.

Sir Frederick Andrewes, emeritus professor of pathology in the University of London, and pathologist to St. Bartholomew's Hospital, perhaps best known for his work on streptococci and their classification (in conjunction with Horder), has died in his seventy-third year.

**Kober Medal Awarded to E. P. Joslyn.**—The Kober Medal of the Association of American Physicians has been awarded to Elliott P. Joslyn, Boston, for his work on diabetes mellitus.

**Pathological Society of Great Britain and Ireland.**—The next meeting of the society will be held at Oxford on July 1 and 2, 1932.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

THE FAMILIAL TENDENCY OF CORONARY DISEASE. J. H. MUSSER and JULIAN C. BARTON, *Am. Heart J.* 7:45, 1931.

The thesis has been advanced that there are two distinct expressions of coronary occlusion. The one is observed in elderly persons, possessors of well marked sclerosis of the arterial tree as a whole, in whom the etiologic factors are those of arteriosclerosis in general and represent largely the effects of senescence. The other occurs in men, as a rule, not past the sixth decade of life who do not have generalized arteriosclerosis, who may have relatively slight, but never exaggerated, hypertension, who have been singularly free from past infections, and who often give a history of coronary occlusion in several members of the family.

AUTHORS' SUMMARY.

HEREDITARY ECTRODACTYLISM IN SIBLINGS. IRWIN J. KLEIN, *Am. J. Dis. Child.* 43:136, 1932.

Inherited deformity of the hand is generally considered to be a dominant trait. Klein reports two cases of ectrodactylism occurring in brothers in whose family there was no history of deformities of the hand. Possibilities as to etiology are presented.

PAUL MERRELL.

REACTIONS FOLLOWING TRANSFUSION OF BLOOD, WITH URINARY SUPPRESSION AND UREMIA. J. BORDLEY, III, *Arch. Int. Med.* 47:288, 1931.

A delayed or prolonged reaction following transfusion is described in seventeen cases. Of these cases, three are reported for the first time, and fourteen have been gathered from the literature. The reaction generally runs a peculiar and highly characteristic course, which presents the following features: Immediately after transfusion, there is a sharp febrile reaction, followed frequently by hemoglobinuria and invariably by suppression of urine. There is an interval of several days during which there is symptomatic improvement but continued oliguria. After this interval, the characteristic features of the delayed reaction develop rapidly. They usually begin with agitation or drowsiness, which is replaced by outspoken evidences of uremia. Convulsions and coma may supervene. The outcome is frequently fatal; eleven of the seventeen patients died. Recovery is associated with diuresis; death occurs in uremia. At autopsy, the kidneys are swollen; the tubular epithelial cells contain droplets of a peculiar pigmented material and show advanced degenerative changes; the tubular lumina are filled with various cells, blood pigment and debris. Small necroses are generally found in the liver. The events may be summarized as follows: A subject receives an injection of incompatible blood, his kidneys are severely damaged, and in due course of time uremia sets in. Several possible explanations of these events are discussed. The delayed reaction is not rare; besides the seventeen cases discussed in detail, a number of cases in the literature are cited.

AUTHOR'S SUMMARY.

PEPTIC ULCER: ASSOCIATION WITH PULMONARY TUBERCULOSIS. MILLS STURTEVANT and L. L. SHAPIRO, *Arch. Int. Med.* 48:1198, 1931.

A review of the literature of the association between peptic ulcer and tuberculosis is given, showing great difference of opinion. Figures obtained from a study of 7,700 necropsies at Bellevue Hospital are presented, which reveal a definitely increased frequency of peptic ulcer in tuberculosis. This frequency is shown to be statistically sound.

AUTHORS' SUMMARY.

MECHANISMS OF THE CONTRACTION AND EVACUATION OF THE GALLBLADDER. LATHAN A. CRANDALL, Arch. Int. Med. 48:1217, 1931.

It is concluded that the liberation of cholecystokinin by the action of fat or of acid in the intestine is the major factor concerned in the contraction and evacuation of the gallbladder that follow a mixed meal. Duodenal motility facilitates this process.

AUTHOR'S SUMMARY.

A SEROLOGIC STUDY OF MULTIPLE SCLEROSIS. A. WEIL and D. A. CLEVELAND, Arch. Neurol. & Psychiat. 27:375, 1932.

Extensive serologic studies on multiple sclerosis convinced Weil and Cleveland that the infectious origin of multiple sclerosis from the "spheres" of Chevassut cannot be sustained; that the serums from patients with multiple sclerosis act destructively on spinal cords of rats much more frequently than normal serums; that an increase of lipase in the serums of patients with multiple sclerosis does not seem to be of diagnostic value, for it also occurs in diseases without concomitant changes in the myelin, and that in the serums of patients with multiple sclerosis inorganic phosphorus is diminished in amount. Multiple sclerosis appears to be due to an endotoxic process.

GEORGE B. HASSIN.

THE MECHANISM OF THROMBOPHLEBITIC EDEMA. L. M. ZIMMERMANN and G. DE TAKÁTS, Arch. Surg. 23:937, 1931.

A series of experiments was made with a view to developing a reliable method for the production of experimental edema of the extremities of dogs. Simple ligation of the iliac and femoral veins, localized chemical phlebitis and periphlebitis and removal of the iliac lymph glands with the retroperitoneal fat and lymphatics from the bifurcation of the aorta to Poupart's ligament failed to produce edema. The injection of 50 per cent to 70 per cent alcohol peripherally into the femoral vein caused extensive venous thrombosis and edema lasting from two and a half to three weeks. Injection of tissue extracts, of dog blood serum and concentrated tissue extract (fibrinogen) also produced venous thrombosis with marked edema. The edema fluid was hemorrhagic and contained a high percentage of protein, indicating damage to the capillary endothelium. If thrombosis by the injection of fibrinogen was prevented by simultaneous injection of heparin, no edema resulted. Hence, thrombosis is a sine qua non for the development of edema. In spite of the marked edema, the injection of india ink at the height of the edema into the foot pads demonstrated that the lymphatic channels were patent, although transportation of the particles to the regional lymph nodes was delayed in the presence of edema. This is explained as being due to the pressure of the fluid on the surrounding tissue. This experimental work demonstrates, therefore, that lymphatic obstruction is not necessary for the production of edema of the extremities in thrombophlebitis, but that the edema results from the thrombosis of the venous channels and damage to the capillary endothelium.

N. ENZER.

THE DISCHARGE OF BILE INTO THE DUODENUM. C. B. PUESTOW, Arch. Surg. 23:1013, 1931.

A method was developed in dogs for the isolation of the loop of duodenum containing the ampulla of Vater to the external abdominal wall, allowing for direct observation of the terminal portion of the common bile duct. In a fasting state no bile was expelled from the ampulla. The orifice of the common duct was usually closed, and the exposed duodenal segment was inactive. The absence of the flow of bile is attributed to the action of a sphincter mechanism at the duodenal end of the duct. The administration of food stimulated the flow of a brown, viscid bile, which began from one to three minutes after the ingestion of food and lasted for several hours. The bile was ejected in spurts and oozes, and was associated with increased activity of the exposed duodenum and hyperemia of the mucosa. After cholecystectomy, the flow of bile was continuous in both

the fasting and the nonfasting states. It was lighter in color and less viscid than the bile that appeared after a dog with a normal and intact gallbladder had been fed. Before the gallbladder had been removed, the intraductal pressure was from 140 to 170 mm. of bile; after removal of the gallbladder it was reduced to from 10 to 20 mm. of bile. Bile salts administered intravenously produced a copious flow of yellow bile. Solutions applied locally to the exposed mucosa of the duodenum occasionally stimulated slight duodenal activity and were rarely followed by the expulsion of a drop of bile.

N. ENZER.

SPONTANEOUS PEPTIC ULCERS OF THE DUODENUM AFTER CONTINUED LOSS OF PANCREATIC JUICE. R. ELMAN and A. F. HARTMANN, Arch. Surg. **23**:1030, 1931.

In dogs from which the pancreatic juice was drained for thirteen days or more, spontaneous peptic ulcer occurred. This would indicate that the pancreatic juice has a neutralizing effect on the gastric acidity and protects the duodenal mucosa.

N. ENZER.

MULTIPLE SCLEROSIS. T. J. PUTNAM, J. B. MCKENNA and L. R. MORRISON, J. A. M. A. **97**:1591, 1931.

Disseminated areas of myelin loss with perivascular infiltration and reactive gliosis may be produced in dogs by the injection of minimal doses of tetanus toxin. The lesions resemble those seen in some cases of human encephalomyelitis. The myelin loss is permanent up to a year from the time of inoculation. The gliosis appears to be progressive. Areas of myelin destruction with reactive gliosis may be produced in dogs by carbon monoxide poisoning. The myelin shows no sign of regeneration within two months. Similar areas of demyelination and gliosis may be produced by embolism with cod liver oil emulsion. Destroyed myelin is not regenerated at the end of five months, but gliosis is progressive. Vascular obstruction appears to play a part in the production of lesions of the two latter types; also perhaps in the first. All three types of lesion resemble closely the "early" plaques of multiple sclerosis. It is not necessary to postulate a specific virus, toxin or ferment to account for the histologic appearance seen in multiple sclerosis.

AUTHORS' SUMMARY.

XANTHOMATOSIS (SCHULLER-CHRISTIAN'S DISEASE; LIPOID HISTIOCYTOSIS). MERRILL C. SOSMAN, J. A. M. A. **98**:110, 1932.

Xanthomatosis (lipoidosis, Schüller-Christian type) is due to a disturbance of lipid metabolism and is characterized by deposits of lipoids, chiefly cholesterol and its esters, in various organs and tissues in the body. The signs and symptoms depend on the location and extent of these deposits. Chief among them are defects in the bones, exophthalmos, diabetes insipidus gingivitis, cessation of growth and occasionally adiposogenital dystrophy.

FROM AUTHOR'S SUMMARY.

FAT TOLERANCE IN EXPERIMENTAL HYPERTHYROIDISM. J. P. SIMONDS and OPAL E. HELPLER, J. A. M. A. **98**:283, 1932.

The total lipid content of the blood plasma is lowered in experimental hyperthyroidism. Feeding 2 cc. of olive oil for each pound of body weight induced a less marked alimentary lipemia in thyrotoxic than in normal animals. Recovery from experimental hyperthyroidism is accompanied by hypercholesteremia.

AUTHORS' SUMMARY.

THE MECHANISM OF GOBLET-CELL SECRETION IN THE MAMMAL: THE EFFECTS OF CYANIDE. H. FLOREY, Brit. J. Exper. Path. **12**:301, 1931.

Oxygen is necessary for the rapid discharge of goblet cells when stimulated. It is suggested that stimulation (any cause of inflammation) results in a stream

of water being passed through the cell. This dissolves the mucin, which is passed out through the open mouth of the cell. Oxygen is necessary for the transport of the water. At the same time it is conceivable that the intracellular mucin becomes more able to deal with the water supplied by altering its power of imbibition. The present experiments do not give any evidence on this matter.

AUTHOR'S SUMMARY.

THE SIGNIFICANCE OF NATURAL VARIATIONS IN THE STRUCTURE AND CORTICAL LIPOID OF THE MOUSE SUPRARENAL. RAYMOND WHITEHEAD, Brit. J. Exper. Path. **12**:305, 1931.

Lipoid is relatively more abundant in the suprarenal cortex of the female than of the male mouse, aged about 150 days. The changes seen after stimulation of the gland were activity of the medulla and sometimes decrease in cortical lipid. The recognition of natural sex differences in the suprarenal gland of the mouse removes the histologic basis for Cramer's theory of self-control. Cramer's interpretations cannot be accepted, since they were made on the assumption that natural variations do not occur in the suprarenal gland of the mouse.

AUTHOR'S SUMMARY.

DEVELOPMENT OF COLLAGEN AND RETICULUM FIBERS IN TISSUE CULTURES. G. MOMIGLIANO-LEVI, Boll. d. Soc. ital. di biol. sper. **5**:891, 1930.

In cultures of connective tissue, extracellular fibers, isolated as well as interlaced, were observed from the first day. These fibers or fibrils were found only in regions in which there were migrated cells.

G. PATRASSI.

EXPERIMENTAL CHRONIC OBLITERATIVE PERICARDITIS. R. FERRARI, Virchows Arch. f. path. Anat. **276**:163, 1930.

Chronic adhesive and obliterative pericarditis was produced in dogs by injecting tincture of iodine into the pericardial cavity. The obliteration of the cavity was followed by signs of severe cardiac insufficiency, such as congestion of the liver, ascites, hydrothorax and subcutaneous edema. These symptoms were much like those of obliterative pericarditis in man and were due to impaired cardiac function.

W. SAPHIR.

THE SUPRARENAL MEDULLA IN VITAMIN B DEFICIENCY. Z. A. SATWORNITZKAJA and W. S. SIMNITZKY, Virchows Arch. f. path. Anat. **276**:342, 1930.

Forty-one pigeons were fed exclusively on a diet of polished rice. They were killed at varying intervals of time and the suprarenal glands compared with those of twelve controls. Vitamin B deficiency caused increased activity of the suprarenal medulla, recognized morphologically by diminution of the chromaffin and pheochrome granules and by vacuolization of the chromaffin cells. The animals on the deficient diet showed a diminished capacity of the cells and tissues to assimilate carbohydrate. Injection of insulin diminished the hyperglycemia and reduced the activity of the suprarenal medulla. From the results in these experiments the inference is drawn that the suprarenal medulla is an important factor in carbohydrate metabolism.

W. SAPHIR.

EFFECT OF LIGATION OF THE RENAL VEIN. M. BRANDT and A. HILSE, Virchows Arch. f. path. Anat. **276**:363, 1930.

Ligation of the renal vein in dogs led to marked venous engorgement of the kidney and to hemorrhages in the medulla and cortex. The capsular veins became greatly engorged and established a partial collateral circulation. Subcapsular hemorrhage was common, and rupture of the capsule with intra-abdominal hemorrhage caused the death of two animals. In time, the hemorrhages within the kidney

were absorbed, the tubules and glomeruli became degenerated and necrotic, and the kidney was transformed into a contracted granular organ, in which function was greatly impaired. If, at this stage, the vein of the other kidney was ligated, death from anuria occurred within four days. Because of the intrarenal hemorrhages that follow ligation of the renal vein and because an adequate collateral circulation is not developed, ligation of the renal vein in man is not recommended.

W. SAPHIR.

EARLY STAGES IN THE CHANGES CAUSED BY IRRADIATED ERGOSTEROL. E. LAAS, *Virchows Arch. f. path. Anat.* **278**:346, 1930.

In Laas' experiments, rabbits were given a single dose of 30 mg. of irradiated ergosterol. This dose uniformly caused the death of the animals on the sixth or seventh day after administration, whether the drug was given by mouth or intravenously. Full grown rabbits were used. The animals were killed at varying intervals of from twelve hours to six or seven days after administration. Calcification of the media of the arteries, which is a change uniformly caused by irradiated ergosterol, begins in the elastic fibrils. No alterations in the elastic fibrils preceding the deposition of calcium could be detected. When larger doses of 300 mg. were used, the localized necrosis of the media of the aorta became diffuse. Calcification of the various internal organs may be preceded by degeneration and necrosis of tissue and is therefore not entirely the result of calcium metastasis.

O. T. SCHULTZ.

### Pathologic Anatomy

ANOMALOUS PAPILLARY MUSCLE ATTACHED TO PULMONARY VALVE. DONALD C. COLLINS, *Am. Heart J.* **7**:79, 1931.

A review is made of the literature on anomalous papillary muscle attached to the pulmonary valve and a case is presented. The patient who died of a disease totally unrelated to the cardiac anomaly, in life made no complaints relative to the heart and was free from cardiac murmurs. Autopsy revealed a thick muscular band, distinct from the myocardium, which arose in the right ventricle and was inserted directly into the base of the anterior cusp of the pulmonary valve.

ALFRED M. GLAZER.

PERSISTENT TRUNCUS ARTERIOSUS. MILO K. MILLER and M. W. LYON, *Am. Heart J.* **7**:106, 1931.

The heart was enormously hypertrophied. Dysphagia was the main symptom calling attention to the defect. Necropsy revealed a persistent truncus arteriosus and an opening from the left ventricle into the right ventricle. Enlarged bronchial arteries functioned as pulmonary vessels. Death occurred at 11 days of age.

AUTHORS' SUMMARY.

TRUNCUS SOLITARIUS AORTICUS. M. A. KUGEL, *Am. Heart J.* **7**:262, 1931.

The reports of two cases of atresia of the pulmonary artery, and other anomalies are presented, with a brief review of congenital heart disease. Of special interest in one of the cases, occurring in a child of 6 months, was the presence of a neuroblastoma of the suprarenal gland with metastasis to the liver.

ALFRED M. GLAZER.

MONONUCLEAR ERYTHROPHAGOCYTOSIS IN THE BLOOD OF A NEW-BORN INFANT. ARTHUR F. ABT, *Am. J. Dis. Child.* **42**:1364, 1931.

The literature on the phagocytosis of erythrocytes by circulating monocytes is here reviewed. This phenomenon was found in a case of anemia of the newborn infant; mononuclear erythrophagocytosis in the circulating blood of a newborn infant has not been previously reported. A brief discussion of the various



theories concerning the origin of monocytes has been given. The futility of attempting to determine the origin of blood monocytes from any type of smear made from the peripheral blood stream alone is pointed out. The phagocytosis of erythrocytes by cells with typical lymphocytic nuclei is reported and pictured.

AUTHOR'S SUMMARY.

AMNIOTIC SAC CONTENTS IN THE LUNGS OF INFANTS. SIDNEY FARBER and LEWIS K. SWEET, *Am. J. Dis. Child.* **42**:1372, 1931.

A study of the lungs of 124 infants who had lived from two hours to five weeks showed that amniotic sac contents were present in the lungs of 88 per cent, and absent in 12 per cent. Large amounts of sac contents were present in 15 per cent of the series. The relation of the aspiration of amniotic sac contents to intra-uterine asphyxia is shown, and the importance of such aspiration as an additional cause of respiratory embarrassment to the new-born infant is emphasized. The methods of recognition of sac contents are given, and the reaction of the lungs to the aspirated materials is described. Two distinct pathologic pictures with intermediate gradations resulting from the aspiration of sac contents are described, and an interpretation of their significance is offered.

AUTHORS' SUMMARY.

HEREDITARY ECTODERMAL DYSPLASIA. DAVID GREENE and HAROLD ABRAMSON, *Am. J. Dis. Child.* **42**:1401, 1931.

A case is presented which, owing to the unusual contour of the skull, the prominent supra-orbital ridges, the broad and flattened nose, the thickened lips, the alopecia of the scalp, the characteristic scanty growth of the eyebrows, the maldevelopment of the nails, the poor teeth and the mental retardation, may be classified in the category of hereditary ectodermal dysplasias. This case and others studied as to etiology seem to point definitely to a congenital or a hereditary factor.

FROM AUTHORS' SUMMARY.

VARIATIONS IN THE PANCREATIC DUCTS AND THE MINOR DUODENAL PAPILLA. SAMUEL SIMKINS, *Am. J. M. Sc.* **182**:626, 1931.

A detailed description of the relationships of the duct of Santorini to the duct of Wirsung is given. A classification is offered of the various arrangements found, as well as a theory to account for such variations. The extraordinary variability of the pancreas in respect to size, shape and ductal arrangement is pointed out. The author has confirmed Opie's finding that in 10 per cent of persons the duct of Santorini is functionally as well as structurally the chief outlet of the external pancreatic secretion, and that such anomalous arrangement may play an important rôle in the prevention or institution of acute hemorrhagic pancreatitis; that it is of great importance likewise in the etiology of chronic interlobular pancreatitis, and that this condition may account for certain puzzling cases of unexplained deaths.

AUTHOR'S SUMMARY.

A PRODUCTIVE-DEGENERATIVE FORM OF ENDARTERITIS OF THE SMALL PIAL VESSELS. D. ROTHSCHILD and K. LOWENBERG, *Arch. Neurol. & Psychiat.* **26**:993, 1931.

In a woman, aged 52, who after a fall complained of pain in the head and back and of "falling spells" and who suffered from hypertension, 250 systolic and 140 diastolic, a combination of pyramidal extrapyramidal and pseudo-bulbar symptoms set in. The condition rapidly led to dementia and death. The necropsy revealed numerous foci of softening in the frontal lobe of the brain, pons, basilar ganglions and cerebellum, which were evidently due to a peculiar widespread vascular lesion. The cells of the intima of the smaller blood vessels, especially of the pia, were greatly swollen and proliferated, resulting in an obstruction of the vascular lumen. The proliferated cells, in the later stages, became degenerated and formed thrombi. The entire wall of the blood vessel was ultimately trans-

formed into a solid connective tissue strand, frequently surrounded by a crescentic hemorrhage. The authors could not identify their observations with anything described—arteriosclerosis, atherosclerosis, syphilis or what is seen in Buerger's disease. It is, they believe, a peculiar form of productive endarteritis, so far not described. The patient also revealed a tumor of the cauda equina, which the authors think was responsible for the paraplegia in flexion which the patient showed in addition to the other signs and symptoms.

GEORGE B. HASSIN.

CHANGES IN THE SPINAL CORD IN LYMPHOGRANULOMATOSIS. ARTHUR WEIL, Arch. Neurol. & Psychiat. 26:1009, 1931.

Weil reports changes in the spinal cord in three cases of Hodgkin's disease with spastic paraplegia. There was a massive lymphogranulomatous tissue over the outer posterior aspect of the dura, which was thickened. The spinal cord showed marked rarefaction and swollen myelin and axons. In a third case, changes were also present in the hemispheres, pons and medulla, in the form of perivascular infiltrations by polymorphonuclear leukocytes, lymphocytes and a moderate number of monocytes. There were also edema around the blood vessels and partial demyelination. The white matter of the spinal cord showed diffuse infiltration of the blood vessels by the same cells as in the brain. Weil also analyzed the forty-three cases reported in the literature, and comes to the conclusion that the central nerve changes in Hodgkin's disease are not caused by a toxin, but are of mechanical origin—an obstruction of the lymphatics or of the blood vessels supplying the spinal cord.

GEORGE B. HASSIN.

EFFECT OF LIVER TREATMENT ON THE CHANGES IN THE SPINAL CORD IN PERNICIOUS ANEMIA. C. DAVISON, Arch. Neurol. & Psychiat. 26:1195, 1931.

Seventeen cases of pernicious anemia complicated by subacute combined degeneration were studied histopathologically. Seven of the patients had been subjected to liver therapy. Clinically, there was apparent improvement in the neurologic signs and symptoms in only two of the treated patients. Histologically, all the treated patients showed progressive glial changes (gliosis). These changes were not observed in the untreated patients with subacute combined degeneration. The myelin sheaths and axis cylinders were not influenced by the liver therapy.

AUTHOR'S SUMMARY.

PROGRESSIVE PALLIDAL DEGENERATION: A NEW CLINICOPATHOLOGIC SYNDROME. N. W. WINKELMAN, Arch. Neurol. & Psychiat. 27:1, 1932.

Winkelman describes a familial condition which was present in two brothers and in which the sole lesion was in the zona reticulata of the substantia nigra and the globus pallidum (these two structures form the so-called pallidum of Cécile Vogt). The clinical picture was rigidity without tremor, chorea or athetosis, absence of oculogyric crisis so typical of postencephalitic rigidity, increased tendon reflexes without signs of pyramidal tract involvement and retinitis pigmentosa. The histologic changes, which were studied in one case, consisted in degeneration of the ganglion cells and demyelination of the pallidum. There was complete preservation of the rest of the brain, corpus striatum and viscera (liver, pancreas and spleen).

On the basis of this case and a few similar cases reported in the literature, Winkelman comes to the conclusion that the condition described by him is like Wilson's disease, a specific morbid entity. He calls it progressive pallidal degeneration, in contrast to Wilson's progressive lenticular degeneration, in which not the pallidum, but the striatum is involved.

GEORGE B. HASSIN.

PERIPHERAL NERVES: ANATOMIC AND PATHOLOGIC CONSIDERATIONS. GEORGE B. HASSIN, Arch. Neurol. & Psychiat. 27:58, 1932.

The changes in peripheral nerves undergoing secondary degeneration have been studied in human and animal material. The main conclusions are that the Schwann

cells and the adjacent endoneural membrane play the main rôle in the removal of the degenerated nerve tissues. Regeneration always originates in the central stump, the new nerve fibers extending along the pathways formed by the endoneural cells. The cells of Schwann cannot be instrumental in this process, as assumed by the antineuronists, for they undergo changes together with the nerve parenchyma with which they are removed to the perineurium or epineurium for final elimination. The absence of such endoneural pathways in the brain or cord is most likely responsible for the lack of central nerve regeneration. The Schwann cells present in the peripheral stump are outgrowths of similar cells from the center whence they accompany or follow the outgrowing nerve fibers. **AUTHOR'S ABSTRACT.**

**ATYPICAL DIFFUSE SCLEROSIS.** K. LÖWENBERG and M. FULSTOW, *Arch. Neurol. & Psychiat.* **27**:389, 1932.

In a patient, aged 26, the disease lasted eleven years. The unusually protracted course was possibly responsible for some uncommon pathologic features. Thus the deeper layers of the white substance including the basal ganglions, anterior commissure and internal capsule were involved; lipoids were sparse; droplets of broken-up myelin invaded the subarachnoid space; the blood vessels and leptomeninges themselves, especially of the cerebellum, often exhibited hyaline degeneration, and the scars in the parenchyma of the brain were a combination of both glia and connective tissues, which were often invaded by lymphocytes, plasma cells and fibroblasts.

**GEORGE B. HASSIN.**

**HYDROCEPHALUS IN AN INFANT WITH VESTIGES OF CHOROID PLEXUS IN THE FOURTH VENTRICLE ONLY.** GEORGE B. HASSIN, *Arch. Neurol. & Psychiat.* **27**:406, 1932.

An infant born with spina bifida developed, one month after birth, a hydrocephalus. This grew steadily larger until death of the infant, three months later. The necropsy revealed a huge hydrocephalus; an occlusion of the third ventricle by an inflammatory process; absence of the choroid plexuses in the lateral ventricles, which after the hardening of the brain were filled with a thick, gelatin-like fluid; a marked purulent ependymitis, and rarefaction and edema of the brain tissue, with progressive and regressive glia changes. The sylvian aqueduct was patent, and the fourth ventricle contained mere traces of a choroid plexus encased within proliferated connective tissue. One of the interesting features in this case was the presence of large quantities of fluids in the lateral ventricles which were deprived of choroid plexuses. This would be inconceivable, were the spinal fluid the product of the choroid plexus.

**AUTHOR'S ABSTRACT.**

**LIPOGRANULOMATOSIS.** M. A. GOLDZIEHER, *Arch. Surg.* **23**:690, 1931.

Three cases of lipogranulomatosis are described; in two the lipogranulomas occurred in the subcutaneous fat of the extremities and the groin and in one in the breast. The term lipogranulomatosis is proposed to replace such terms as fat necrosis, oleogranuloma, cystic lymphangitis and others. The pathogenesis of this lesion, whatever the etiology, is uniform. Primary necrosis of fat is followed by the formation of granulation tissue. The liberation of free fatty material, cholesterol crystals and fatty acids stimulates the formation of giant cells and the proliferation of epithelioid cells. Calcium soaps are formed in the areas of necrosis and may eventually develop into solid calcification. Liquefaction of the liberated fat frequently results in small cysts, the walls of which are composed of fibrotic granulation tissue. Trauma seems to be an etiologic factor, but inflammation and circulatory disturbances account for some of the cases. Others appear to be spontaneous. The immediate initiating factor is the liberation of lipase from the injured fat.

**N. ENZER.**

THE STRUCTURAL CHANGES IN INFLAMMATORY MUCOUS SECRETION IN THE CAT. H. FLOREY and R. A. WEBB, *Brit. J. Exper. Path.* **12**:286, 1931.

A description is given of the histologic changes involved in the rapid evacuation of goblet cells in the colon of the cat. The histologic appearance of the mucosa following the application of mustard oil varied in degree with the concentration of the oil and the length of time of application. With the egress of concentrated mucus through the stroma of the goblet cell the nucleus and cytoplasm of the latter assume the appearances of a columnar epithelial cell. With a continuance of the irritation the cell appears to lose cytoplasm and becomes cuboidal and finally very like squamous epithelium. This applies equally to the nonglobular epithelial cells present. Reformation of the goblets in the colonic mucosa takes place with considerable rapidity. It is suggested that the goblets arise from old mucin-producing cells elaborating new secretion. The cells at the bases of the crypts undergo discharge and exhibit their cycle of changes before those near the surface. The mucus-secreting cells of the stomach are more resistant to irritation than those of the colon.

C. E. RICHARDS.

THE PATHOLOGIC OCCURRENCES IN THE LIVER IN EXPERIMENTAL VENOUS STAGNATION. C. BOLTON and W. G. BARNARD, *J. Path. & Bact.* **34**:701, 1931.

When any obstacle is interposed to the return of blood through the inferior vena cava to the heart, whether it is due to failure of the right side of the heart from one of its numerous causes or to increased pressure in the chest in any of its forms, the resulting increase of venous pressure passes back through the liver to the portal vein, but, owing to distention of the capacious splanchnic area, a high portal pressure sufficient to maintain compensation is impossible and the arterial pressure fails. Arterial constriction does not relieve the condition. At this stage there is marked venous stagnation in the liver with resulting necrosis and cellular degeneration; there is an increase in the production of lymph, and the lymph stagnates in the liver and leaks out of the capsule as ascites. The other organs, to which the liver acts as a buffer, do not show such extensive changes; their capillaries are congested, and excessive production of lymph leads to dropsical effusion. At a later stage, the blood increases in volume, and the pressures go up in all parts; the flow of blood through the liver and the flow of lymph from its lymphatics are increased. In this way, compensation is partially effected, and in local obstruction of the inferior vena cava anastomoses, more readily established in the systemic than the portal area, complete the process of compensation, although the liver contains permanently dilated channels around the hepatic venules. In the condition of congestive heart failure, recovery of the heart entirely removes the obstruction, and compensation is completely restored; after repeated attacks, the liver and great veins remain permanently dilated. Any subsequent necrosis of the cells must be looked on as the result of acute exacerbation of the congestion. At first sight it appears anomalous that a higher portal pressure should be associated with less severe cellular change in the liver. But such is not the case. In 1904 it was proved by one of us that raised intracapillary pressure was merely a contributing factor in the causation of passive edema and had not the importance usually ascribed to it. The same is true of these changes in the liver. If it were possible to raise the portal pressure high enough to ensure a normal flow of blood through the obstruction, the changes in the liver would be limited to dilatation of the vessels and a minimal degree of pressure atrophy.

AUTHORS' SUMMARY.

PULMONARY ASBESTOSIS IN A DOG. N. H. SCHUSTER, *J. Path. & Bact.* **34**:751, 1931.

The lungs of a dog suffering from asbestosis are described. The histology was typical, but naked asbestos fibers were present instead of the asbestos bodies usually found, and the case therefore throws no direct light on the mode of formation of these bodies. Asbestos bodies do not alter the course or nature of the disease. The fibrosis is probably a result of the toxic action of the asbestos, a combined silicate, and not a mechanical effect.

AUTHOR'S SUMMARY.

RENAL CHANGES IN POISONING WITH MERCURIC CHLORIDE. G. PATRASSI, Clin. med. ital. **61**:76, 1930.

Thirteen instances of various grades of mercuric chloride poisoning have been studied with particular reference to the changes in the kidney. The gravest and most typical renal lesions were found in the cortical tubules, in which there was an atypical new formation of nuclei of the epithelial cells with deposits of calcium in the cytoplasm. This change is attributed to a toxic stimulative action of the poison.

G. PATRASSI.

ALTERATIONS OF THE PANCREAS IN HEART DISEASE. F. GERLEI, Virchows Arch. f. path. Anat. **276**:148, 1930.

The pancreas was studied histologically in thirty cases of death due to heart disease of various kinds. When thrombi are present in the left side of the heart, embolic infarction may occur in the pancreas as in other organs. In decompensated heart disease, characteristic alterations may occur in the pancreas, the changes being due to congestion of the organ itself and to congestive duodenitis. Arrest of pancreatic secretion follows, and this may lead to multiple necroses. Arrest of secretion may also be followed by hypertrophy of the islands of Langerhans. Metaplasia of the epithelium of the ducts was noted not infrequently.

W. SAPHIR.

ENDOCARDIAL CALCIFICATION IN DOMESTIC ANIMALS. W. S. TSCHERNIAK and N. ROMANOV, Virchows Arch. f. path. Anat. **276**:170, 1930.

In 9 cases of calcification of the parietal endocardium in 238 horses and 3 dogs, the process attacked chiefly the endocardium of the left ventricle and was associated with calcification of the walls of the blood vessels and with a degenerative process of the endocardium in general.

W. SAPHIR.

PLASMA CELLS IN INFECTIOUS GRANULOMAS. W. ADAMOWICZ, Virchows Arch. f. path. Anat. **276**:230, 1930.

Adamowicz directed his attention to the plasma cells in various kinds of infectious granulomas. The cells are derived chiefly from the lymphocytes of either hematogenous or histiogenic origin. Their presence bears no relation to the character of the granulomatous tissue, but is apparently dependent on local conditions in the tissues and on the duration of the process. Vacuolar degeneration of the nuclei and abnormal staining reactions of the nucleoli were noted. Pigment phagocytosis by plasma cells was occasionally seen and is considered a sign of selective activity. The accumulation of plasma cells in the granulation tissue is looked on as a protective mechanism to wall off the morbid process.

W. SAPHIR.

GENESIS OF EMPHYSEMA OF THE LUNG. N. A. PODKAMINSKY, Virchows Arch. f. path. Anat. **276**:279, 1930.

The author attaches great importance to abnormality of the pleura in the development of emphysema of the lung. Under normal conditions, distention of the lung during inspiration is arrested by the pleura. When portions of the pleura become altered, the involved pleura can no longer limit distention of the pulmonary tissue. The interalveolar septums of the adjacent lung tissue are insufficiently supported and are compressed by overdistention of the alveoli. The septal capillaries are compressed, and the blood supply of the septums is diminished. Atrophy and disappearance of septums follow, resulting in the characteristic picture of alveolar emphysema. The emphysema of childhood may be due to congenital maldevelopment of the pleura. Occupational emphysema is usually the result of pneumoconiosis, and is therefore a vicarious and not an essential emphysema, although constitutional factors may play a part in this form of emphysema. After examination of more than 450 baggage carriers, the author is convinced that heavy physical labor alone is not able to cause emphysema.

W. SAPHIR.

RENAL ARTERIOLOSCLEROSIS AND HYPERTENSION. M. A. ZACHARJEWSKAJA, *Virchows Arch. f. path. Anat.* **276**:380, 1930.

Forty-four cases of hypertension, with and without clinical evidence of arteriosclerosis of the kidneys, were studied clinically and at autopsy. The renal vascular changes noted were intimal hyperplasia of the larger vessels and hyalinization and fatty infiltration of the arterioles. Muscular hypertrophy of the media of the arterioles was not seen; on the contrary, the muscular wall became thinner with advancing years. Proportional to the degree of vascular change, the glomeruli and tubules undergo degenerative reactions, such as hyalinization, fatty change and cicatrization. No relation could be determined between the degree of renal insufficiency and the number of glomeruli destroyed, since other factors, such as functional vascular alterations, vascular tone and cardiac efficiency, play an important part in the renal insufficiency. There also was no definite relation between the degree of cardiac hypertrophy and alterations of the renal arterioles. Cardiac hypertrophy was noted in hypertension without renal arteriolar changes, and was sometimes absent in cases with moderate arteriolar change.

W. SAPHIR.

EUNUCHOIDISM. F. ALTMANN, *Virchows Arch. f. path. Anat.* **276**:455, 1930.

From a thorough morphologic study of eleven cases of eunuchoidism Altmann concludes that the signs and symptoms of this condition are directly due to diminished or abolished gonadal function. The hyperplastic and hypertrophic cellular changes frequently seen in the pituitary gland are nonspecific and not characteristic, although the acromegalic alterations sometimes seen in eunuchoidism may be the result of these pituitary changes. Careful examination of all the ductless glands failed to reveal any definite alterations except in the sex glands.

W. SAPHIR.

### Microbiology and Parasitology

ENDEMIC PURPURIC MENINGOCOCCUS BACTEREMIA IN EARLY LIFE. STAFFORD MCLEAN and JOHN CAFFEY, *Am. J. Dis. Child.* **42**:1053, 1931.

In eighteen cases of meningococcus bacteremia with purpura, meningococci were demonstrated in smears from the purpura in fifteen, or 83 per cent. In four, or 12.5 per cent, of thirty-two cases of meningococcus bacteremia with purpura no inflammatory changes were demonstrated in the cerebrospinal fluid. The mortality in meningococcus bacteremia with purpura was 50 per cent. All patients younger than 6 months of age died. The mortality in the first year of life was 66.6 per cent. Hemorrhage into the suprarenal glands was present in three cases. Suprarenal damage is a possible cause of death in rapidly fatal cases. Of seven cases in which autopsy was adequate for this determination, enlargement of the thymus gland and hyperplasia of the lymphoid tissue of the intestine were present.

Smears from the purpuric skin lesions in meningococcus bacteremia offer a rapid, exact and convenient method for immediate bacteriologic diagnosis. In 12.5 per cent of cases it was the only method by which an immediate bacteriologic diagnosis could be made.

AUTHORS' SUMMARY.

COCCIDIOIDAL DERMATITIS. E. P. ZEISLER, *Arch. Dermat. & Syph.* **25**:52, 1932.

A widespread dermic infection with *Coccidioides immitis* is described, presenting a combination of nodular, crusted, granulomatous lesions resembling mycosis fungoides, with lichenoid papules simulating miliary lupus, and pigmented, atrophic, annular scars. In later stages, the papillomatous and verrucous character of the eruption gave rise to a close clinical resemblance to blastomycosis. The chronic nature of the dermic lesions and the absence of demonstrable subcutaneous, glandular, pulmonary or osseous lesions suggest the term chronic coccidioidal dermatitis. The skin as the portal of entry of the infection and the possibility that animals may

be carriers of the disease are suggested by the history of the case. Histologic changes in the form of nodules in tissue surrounding blood vessels suggest dissemination by the blood stream.

EDNA DELVES.

CULTIVATION OF *BACILLUS LEPRAE* AND EXPERIMENTAL LEPROUS LESIONS IN MONKEYS. E. B. MCKINLEY and M. H. SOULE, J. A. M. A. **98**:361, 1932.

The experiments described include (1) the experimental production of granulomatous lesions suggestive of early lesions of leprosy in two species of monkeys by intradermal inoculation of human leprosy material; (2) the cultivation of acid-fast bacilli (presumably *B. leprae*) from the nodules of human leprosy on several artificial mediums in various gaseous environments, and (3) the experimental production of granulomatous lesions suggestive of early leprosy in two species of monkeys by intradermal inoculation of cultures of acid-fast bacilli from human leprosy material grown on artificial mediums. We believe that the experiments indicate a step forward in the fulfilment of Koch's postulates for the causative agent in the disease of leprosy.

AUTHORS' SUMMARY.

LABORATORY INFECTION WITH SYPHILIS. G. E. WAKERLIN, J. A. M. A. **98**:479, 1932.

A laboratory assistant accidentally received a puncture on the back of the right wrist by a needle attached to a syringe containing a suspension of *Spirochaeta pallida* prepared from syphilitic rabbit testicular tissue. The strain of *Spirochaeta pallida* in question had been carried in rabbits for thirteen years. Four weeks after the puncture a light arthritis of the wrist joint was observed, and five weeks later a typical macular syphilitic eruption with a four plus Wassermann reaction of the blood developed. There seems to be no question but that this is a case of true laboratory infection.

BACTERIOPHAGE ADAPTATION. P. J. BEARD, J. Infect. Dis. **49**:367, 1931.

Much of the discussion in the literature concerning adaptation is based on errors in technic and interpretation. The experimental work described in this paper has revealed no evidence of adaptability within the limits of the definition formulated. If this definition is acceptable, then by d'Herelle's own criteria of life the bacteriophage is not living, since it does not possess the faculty of adaptation. The fact that a substance lacks certain of the properties of living matter does not eliminate the possibility that it may be endowed with other attributes of that state. It is not impossible that the bacteriophage may be of such a nature.

AUTHOR'S CONCLUSIONS.

MYCOBACTERIUM (SP?) ISOLATED FROM PLEURAL EXUDATE. P. W. BEAVEN and S. BAYNE-JONES, J. Infect. Dis. **49**:399, 1931.

In an attempt to classify this organism the authors feel justified in expressing the opinion that it is neither a true tubercle bacillus nor an atypical tubercle bacillus. The organism was found in abundance in the pleural fluid of the patient. The violent cutaneous reaction to a filtrate of the organism suggests its relationship to the pulmonary infection of the patient. It was less pathogenic for guinea-pigs than for the patient, but the pulmonary lesions in both cases had much in common. The organism described was found to have characteristics in common with, as well as significant differences from, saprophytic acid-fast bacteria.

EDNA DELVES.

DIPHTHEROID PHASE OF STREPTOCOCCI. L. B. JENSEN and H. B. MORTON, J. Infect. Dis. **49**:425, 1931.

A strain of bacteria isolated from the urine of a patient suffering from cystitis could be made to mutate at will into streptococcal forms or into diphtheroid-like forms. On blood agar, diphtheroidal types were regularly produced, whereas in

dextrose brain broth the strain revealed pure cultures of streptococci. In the diphtheroid phase, the bacteria were avirulent when tested by intravenous injection into rabbits; in the streptococcal phase, the bacteria were virulent and had elective localizing power. On blood agar, cultures of organisms from the kidneys of rabbits in which elective localization had been demonstrated revealed only diphtheroid-like forms, whereas in dextrose brain broth the cultures yielded only streptococci, as revealed on examination of stained smears. Tests for agglutination and the absorption of agglutinins disclosed that the diphtheroid and streptococcal phases were antigenically distinct. Tests for the production of peroxide indicated that in the diphtheroid phase the strain was a feeble producer of peroxide, and that on repeated subculture on blood agar it lost this property. Studies of a strain of bacteria isolated from the blood of a patient suffering from subacute bacterial endocarditis revealed a similar phenomenon.

AUTHORS' SUMMARY.

PHENYL-MERCURY-NITRATE AS A DISINFECTANT. L. A. WEED and E. E. ECKER. J. Infect. Dis. 49:440, 1931.

High dilutions of an aqueous solution of phenyl-mercury-nitrate exert a strong disinfecting and antiseptic power on bacteria and molds. The bactericidal power of a solution of phenyl-mercury-nitrate is not in the nature of a selective action and is not inhibited in the presence of tissues or urine. An aqueous solution may be given in large doses orally, intravenously or intraperitoneally, with no serious effects. This substance is a powerful, safe and practical disinfectant for general and specific uses, since it has no odor, color, taste or corroding capacities and does not stain.

AUTHORS' SUMMARY.

ELECTRICAL BEHAVIOR OF LEISHMANIA DONOVANI. A. J. SALLE, J. Infect. Dis. 49:450, 1931.

*L. donovani* migrated to the cathode at  $pH$  2.16 and below. At from  $pH$  3.1 to  $pH$  10.08 the organisms wandered to the anode. The iso-electric point fell between  $pH$  2.16 and  $pH$  3.1. The great majority of bacteria have an iso-electric point at about  $pH$  3. The results show that *L. donovani* behaves electrically like the bacteria.

AUTHOR'S SUMMARY.

VARIATION OF A B. COLI-LIKE ORGANISM. W. J. NUNGESTER and S. D. ANDERSON, J. Infect. Dis. 49:455, 1931.

The authors describe a series of variants which were produced from a *B. coli*-like organism isolated from a case of empyema of the gallbladder. These variants differed in form of colony, with colonial structures of the R, S and intermediate types. A mucoid form of colony is also described. Marked difference in the ability of the variants to ferment lactose is noted when such studies are carried out on solid mediums. All variants, except the mucoid, are agglutinated in a high dilution of the agglutinating serum of the parent form. Changes from lactose-fermenting to the nonlactose-fermenting forms is effected with difficulty. The reverse change is readily brought about.

FROM AUTHORS' SUMMARY.

NEW MEDIUMS FOR, AND THE METABOLISM OF, LEISHMANIA DONOVANI. A. J. SALLE, J. Infect. Dis. 49:473 and 481, 1931.

The new solid medium yields large quantities of organisms for inoculation experiments and the preparation of vaccines. The liquid medium permits the study of the metabolism of the blood organism.

The results of metabolic studies show that in the absence of dextrose there is a great increase in the ammonia nitrogen fraction in the medium. The  $pH$  value also shows a large increase. In the presence of dextrose there is a minimum of activity on the nitrogenous constituents of the medium. The presence of a utilizable carbohydrate exerts a marked sparing action on the protein constituents of the medium.

FROM AUTHOR'S SUMMARY.



LETHAL RATES FOR PORCINE STRAINS OF BRUCELLA ABORTUS. R. A. BOAK and C. M. CARPENTER, *J. Infect. Dis.* **49**:485, 1931.

The results indicate that the present requirements for the pasteurization of milk (heating at from 142 to 145 F. for from twenty to thirty minutes) are adequate for destroying the most virulent strains of *Br. abortus*.

AUTHORS' SUMMARY.

BACTERIOLOGY OF THE BLOOD IN INFECTIOUS ARTHRITIS. H. BERNHARDT and P. S. HENCH, *J. Infect. Dis.* **49**:489, 1931.

Eighty blood cultures were made from twenty patients with chronic infectious arthritis. Three different methods of cultivation were used; one of them was that published by Cecil, Nicholls and Stainsby. Streptococci were not found in any culture. Thus we have been unable to confirm the results of these authors.

FROM AUTHORS' SUMMARY.

ACID-FAST BACTERIA IN SOILS. C. A. FREY and W. A. HAGAN, *J. Infect. Dis.* **49**:497, 1931.

Using a technic devised by Söhngen, we have been able to demonstrate the presence of acid-fast bacteria in one hundred samples of soils collected from various parts of the United States. A technic is described by the use of which many, if not all, of these organisms may be isolated in pure culture. The organisms isolated in pure culture present a diversity of cultural features, in many instances resembling those of well known organisms. They have been grouped into three rough groups on the basis of cultural features. By means of cutaneous tests on sensitized or immunized guinea-pigs it has been shown that the organisms of any one of these groups exhibit a closer relationship to one another than to the organisms of the other groups.

AUTHORS' SUMMARY.

SYPHILIS OF THE PULMONARY ARTERY. M. SINDONI, *Arch. ital. di anat. e istol. pat.* **1**:629, 1930.

In fifty-nine cases of syphilitic aortitis, the pulmonary artery showed syphilitic lesions in eight. These lesions consisted in perivascular lymphocytic infiltrations in the adventitia, especially in the part in contact with the aorta. This distribution suggested that the lesion in the pulmonary artery was due to direct extension from the aorta.

G. PATRASSI.

THE EFFECT OF TOXIC SUBSTANCES OF THE TUBERCLE BACILLUS ON THE LIVER. A. M. LEWIN, *Virchows Arch. f. path. Anat.* **276**:101, 1930.

Lipoid and albuminous endotoxic substances of the tubercle bacillus were injected into rabbits, and the changes in the liver and spleen studied at intervals varying from ten days to twelve months. No histologically specific tuberculous changes were found. In the liver there occurred a connective tissue hyperplasia that led to a cirrhosis much like the common interlobular cirrhosis of man. In the spleen the connective tissue was increased, and the sinus endothelium was hyperplastic. The findings support the view that tuberculous intoxication may play an important rôle in the pathogenesis of interlobular cirrhosis.

W. SAPHIR.

PROTOZOON-LIKE INCLUSIONS IN AN INFANT. A. JACUBOWICZ, *Virchows Arch. f. path. Anat.* **276**:279, 1930.

In an infant who died six days after birth, with the symptoms and clinical diagnosis of congenital syphilis, protozoon-like bodies were found in the epithelial cells of the kidney, liver, pancreas and lungs, and in mesenchymal cells of the umbilicus and of the walls of the bronchi. The changes in the tissues were like those of congenital syphilis, but the Wassermann reaction was negative and spiro-

chetes could not be demonstrated in the tissue. In spite of the histologic similarity to syphilis, the author believes that the changes may not have been due to syphilis, but that the protozoon-like bodies were the cause of a disease hitherto unknown.

W. SAPIHR.

AGRANULOCYTOSIS. M. BROGSITTER and H. VON KRESS, *Virchows Arch. f. path. Anat.* **276**:768, 1930.

This is a critical survey of the literature, with a report of personal cases. The authors believe that the view that agranulocytosis is a well characterized and definite disease entity, which gained acceptance following W. Schultz's description of the syndrome and is still held by many, must be abandoned. Many bacterial and toxic agents can produce an identical picture under conditions as yet not well understood.

W. SAPIHR.

EXPERIMENTAL TUBERCULOSIS OF THE SKIN. S. S. VAIL, *Virchows Arch. f. path. Anat.* **277**:115, 1930.

In 108 experiments, tuberculous material was introduced into the skin of guinea-pigs, rabbits and monkeys. The material used was cultures of the tubercle bacillus of different degrees of virulence, emulsions of human skin affected by lupus and emulsions of organs of infected guinea-pigs. The intradermal inoculation resulted in the production of a skin disease very similar in its histologic characteristics to human lupus. The tuberculous process became generalized comparatively late and ran a more chronic course than that which follows the usual modes of inoculation. The more prolonged course of the generalized infection was characterized by absence of caseation and by a tendency to productive inflammation and cicatrization.

W. SAPIHR.

ACTINOMYCOSIS OF THE STOMACH AND LIVER. S. DERISCHANOFF, *Virchows Arch. f. path. Anat.* **277**:130, 1930.

The author reviews the literature of primary actinomycosis of the stomach and presents two cases of his own. In one of these, the patient was operated on shortly before death because of hematemesis and acute abdominal symptoms. The portal of entry in each case was apparently the stomach, the liver being secondarily infected by way of the blood stream. The histologic picture was the usual one of suppuration, proliferation of granulation tissue and scar formation. The tissue contained the typical actinomycetic granules, but in small numbers. The predominance of epithelioid cells in some areas was striking.

W. SAPIHR.

THE ETIOLOGY OF PERICARDITIS. A. A. GERKE, *Virchows Arch. f. path. Anat.* **278**:1, 1930.

For his study Gerke used 75,856 clinical and 26,771 necropsy protocols of the past twenty years. These were taken from institutions in Moscow that had been under the direction of the same school of pathology. The material included 4,442 records from a hospital that received chiefly cases of sepsis. In this large material there were 1,756 cases in which pericarditis was found. The author summarizes these cases as follows: rheumatic infection (including valvular and myocardial lesions presumably due to rheumatic infection), 336 cases, or 19.1 per cent; tuberculosis, 275 cases, or 15.6 per cent; pneumonia and pneumococcal pleuritis, 251 cases, or 14.2 per cent; nephritis, 151 cases, or 8.6 per cent; sepsis, 301 cases, or 11.4 per cent; miscellaneous conditions, 411 cases, or 23.1 per cent; conditions the etiology of which was unknown, 43 cases, or 2.4 per cent. Cases of simple hydro-pericardium are not included. The interesting data relating to the incidence of pericarditis in various disease groups can be only briefly indicated. In 1,634 cases of pneumococcal infection in which necropsy was performed, there were 334 examples of pericarditis. The latter condition was associated more often with pneumonia of the lower lobes than with that of the rest of the lung, and most frequently with

consolidation of the anterior part of the left lower lobe. In 128 cases of influenza in which necropsy was performed, pericarditis was detected 23 times. The influenza bacillus was not isolated in any case, a fact that the author thinks may be due to the hurried technic of the war years, during which period most of the necropsies were done. The relative infrequency of pericarditis in sepsis is indicated by the occurrence of 149 pericardial involvements in a total of 5,359 cases of erysipelas, scarlet fever and septicemia, the latter chiefly of genital origin in women. In contrast to this is the high incidence of pericarditis in purulent tonsillitis; of 10 fatal cases coming to necropsy, all showed pericarditis; in addition, pericarditis was detected clinically 36 times in cases of tonsillitis that did not come to necropsy. Pericarditis was seen 81 times at necropsy in association with cancer, but was detected clinically in only 7 of 2,214 cases of tumor. Gummatous pericarditis was found in 12 of 28 cases in which death was ascribed to syphilis. The rarity of pericarditis as a complication of certain diseases is shown by its occurrence only once in 26,771 cases of gonorrheal infection and not once in 4,010 clinical and 639 cases of typhoid that came to necropsy.

O. T. SCHULTZ.

SPREAD OF RABIES VIRUS WITHIN THE BODY. F. SCHWEINBURG and F. WINDHOLZ, *Virchows Arch. f. path. Anat.* **278**:23, 1930.

Experimental and histologic evidence has established without question the transfer of rabies virus from the point of introduction to the central nervous system by way of the nerve paths. Whether the virus may not also be transported by the blood stream, for which some evidence has been offered, has not been determined in equally definite manner. To settle the question, the authors used the method of parabiosis. That a communication between the circulations of two united rats has occurred by the sixth day after union could be proved by the subcutaneous injection of an aqueous solution of methylene blue (methylthionine chloride, U. S. P.) into one of the pair. The dye appeared in the urine of the other member of the pair as soon as in that of the animal receiving the injection. Circulatory union having been proved in this manner, rabies-fixed virus was injected intramuscularly into the thigh of one of the parabiotic rats. In each of eighteen experiments, rabies virus could be demonstrated only in the animal receiving the injection.

O. T. SCHULTZ.

HISTOLOGY OF COCCIDIOIDAL GRANULOMA. R. H. JAFFÉ, *Virchows Arch. f. path. Anat.* **278**:42, 1930.

Because the disease has thus far been limited to the western hemisphere, Jaffé presents a study of two cases of coccidioid granuloma observed at the Cook County Hospital, Chicago. Localization of the organism, *Cryptococcus* (*Coccidioides*) *immitis*, is followed by the development of miliary epithelioid nodules that do not tend to coalesce with each other and are separated by a dense infiltration of plasma cells. The cyst develops and ripens within a giant cell at the center of the nodule. Rupture of the cyst is followed by destruction of the giant cell and by infiltration by leukocytes, transforming the epithelioid nodule into a miliary abscess. In the skin, the histologic character of the granulation tissue is soon disturbed by secondary infection. The development of the miliary nodules about the capillaries leads Jaffé to conclude that infection of the skin occurs by way of the blood stream. In the experimental lesions of guinea-pigs, the characteristic epithelioid or histiocytic reaction is absent, the lesions being purulent from the beginning.

O. T. SCHULTZ.

ALVEOLAR ECHINOCOCCUS DISEASE IN GENÈVE. F. KLAGES, *Virchows Arch. f. path. Anat.* **278**:125, 1930.

Echinococcus disease is more prevalent in Genève than in any other Swiss canton. Most of the cases have been of the hydatid form, but a few cases of the multi-locular or alveolar form have also been encountered, disproving Posselt's assertion

of the strictly distinct geographic distribution of the two forms. Klages presents a detailed histologic study of a case of primary alveolar echinococcus disease of bone. He discusses the condition at some length because of its rarity. He accepts only two previously reported cases of primary alveolar echinococcus disease of bone. Two additional previously reported cases he believes to have been primary in the liver. In bone, the alveolar form of the disease causes great destruction of bone, necrosis and inflammation of the marrow, and extra-osseous invasion. He believes that the condition may be mistaken for tuberculous caries of bone. From the latter, echinococcus disease is differentiated by the absence of caseation and by the presence of leukocytic infiltration.

O. T. SCHULTZ.

POSTVACCINIAL ENCEPHALITIS. A. ESSER, *Virchows Arch. f. path. Anat.* **278**:200, 1930.

Esser reports two cases of acute cerebral involvement following vaccination against smallpox, in infants, aged, respectively,  $3\frac{1}{2}$  and  $1\frac{3}{4}$  years. Fever and cerebral symptoms developed on the sixth and seventh days following vaccination, which was a first vaccination in each case. In the older child the histologic changes were those usually found in postvaccinial encephalitis: localized myelin sheath degeneration leading to complete demyelination, focal glial proliferation and perivascular lymphocytic infiltration. In the younger child perivascular infiltration was absent, and changes of the myelin sheaths were very slight. There was, however, marked diffuse reaction of the glia and ganglion cells, the latter exhibiting varying degrees of degeneration. Inoculation of material from the younger child into rabbits had negative results. Negative results in experiments on animals do not warrant the conclusion reached by some that the vaccine virus itself cannot be the cause of the cerebral damage that may follow vaccination. Esser believes that in spite of negative results in inoculation of experimental animals, the vaccine virus may be the cause of the encephalitis.

O. T. SCHULTZ.

REACTION OF LYMPH NODES IN EXPERIMENTAL INFECTION. A. SJÖVALL and HELGE SJÖVALL, *Virchows Arch. f. path. Anat.* **278**:258, 1930.

Helmann, in whose laboratory at the University of Lund this work was done, has maintained that the so-called germinal centers of the lymphoid tissues are not the sites of formation of lymphocytes, as postulated by Flemming, but are hyperplastic reticulo-endothelial reaction centers that have an important rôle in infection and immunity. In this work, a study was made of the reaction of the popliteal nodes of the rabbit to infection produced by the subcutaneous injection of virulent *B. pyocyaneus* in the popliteal region. The nodes of the opposite popliteal region served as controls, as did also the nodes of both sides in normal rabbits. The reaction centers were sketched in low magnification projection drawings of serial sections. An increase in the number of reaction centers in the nodes of the infected side was apparent on the fifth to seventh days after inoculation and reached its height in from ten to twenty days. At the end of a month regression of the hyperplastic centers was evident. Although an increase in the size of the centers was evident, the most striking change was an increase in their number, exceeding by hundreds those of the control nodes.

O. T. SCHULTZ.

HISTOLOGY OF LESIONS CAUSED BY THE CALMETTE AVIRULENT (BCG) TUBERCLE BACILLUS. C. SCHILLING, *Virchows Arch. f. path. Anat.* **278**:462, 1930.

Guinea-pigs were inoculated intravenously or intracardially with the Calmette bacilli and killed at intervals of from seven to sixty-one days. One animal of each series was superinfected with a virulent strain of human tubercle bacillus fourteen days after the primary inoculation in one instance and forty-seven days after in the other instances. In each series, animals were infected with the virulent strain without previous inoculation of the avirulent strain. The Calmette organ-

isms localize in the lungs and multiply with the formation of dense bacillary clumps, in which the bacilli in time lose their acid-fast property. They evoke the formation of minute lesions grossly like typical miliary tubercles. These are composed of endothelioid cells, with an outer zone of fibroblasts. The lesion is infiltrated by polymorphonuclear leukocytes, many of which undergo degeneration. Lymphocytic infiltration is inconspicuous. Typical Langhans' giant cells are only occasionally formed, and caseation necrosis does not occur. The lesions become encapsulated by connective tissue and may finally disappear without leaving a trace. The organism may not be considered wholly avirulent, since it is able to multiply in the tissues and to evoke a tissue reaction in which necrosis of leukocytes occurs. In animals superinfected with virulent tubercle bacilli, typical tubercles were not formed and multiplication of the injected bacilli could not be detected. Schilling concludes that there can be no question of the antigenic action of the Calmette bacillus against virulent bacilli.

O. T. SCHULTZ.

#### EXPERIMENTAL TUBERCULOSIS AND THE RETICULO-ENDOTHELIAL SYSTEM.

M. M. SCHEININ and J. M. PEISSAKHOVITSCH, *Virchows Arch. f. path. Anat.* **278**:623, 1930.

A series of guinea-pigs infected by the subcutaneous inoculation of an emulsion of human tubercle bacilli was vitally stained with trypan blue. Subcutaneous injection of the dye was found to be preferable to intraperitoneal injection. The dye was injected once or twice a week. Another series of infected animals was subjected to the subcutaneous injection of 0.5 per cent colloidal silicon every five to seven days. A third series received trypan blue followed by colloidal silicon. Trypan blue caused the usual marked hyperplasia of the reticulo-endothelial cells, which were filled with stored dye. These cells wandered in numbers into the developing tubercles, but because their functional activity was apparently interfered with by the ingested dye, they quickly died and went to pieces. Only an occasional epithelioid cell of the tubercle ingested dye. Infected animals treated with trypan blue did not live as long as untreated infected animals. The average duration of life of the latter was about twice that of the animals treated with trypan blue. Animals treated with colloidal silicon alone lived longer than the control animals. There was greater tendency to fibrosis of the tuberculous lesions in the former than in the latter. Animals receiving trypan blue followed by silicon lived no longer than those receiving trypan blue alone. Histologically, a slightly greater tendency to the formation of connective tissue about the tubercles was noted.

O. T. SCHULTZ.

### Medicolegal Pathology

TAR CANCER IN MAN. G. BETTAZZI, *Arch. ital. di chir.* **30**:45, 1931.

This article contains a review of the literature on tar cancer in man, also an account of a survey of Italian statistics and observations bearing on tar cancer. Three cases are described.

TRAUMA AND SCHIZOPHRENIA. R. NEUSTADT, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18**:1, 1931.

Psychic disturbances of a general nature may follow injuries to the brain. But it is difficult to state whether schizophrenia may develop in direct connection with a cerebral traumatism, since the causal and pathogenic factors of schizophrenia are still obscure. Theoretical explanations and discussions in a general way are of no avail; each particular case must be considered individually and analyzed clinically as to its psychiatric merits. Two clinical cases are presented, and the differential diagnostic points relating to traumatic pseudodementia, traumatic psychosis or degenerative psychosis following injury are discussed. A schizophrenia-like symptom complex may occur in instances of trauma to the brain and is termed symptomatic schizophrenia, which is practically very difficult, even impossible, to

differentiate from the genuine, or heredodegenerative, schizophrenia. The author concludes that in every instance in which a positive diagnosis of genuine schizophrenia was made its causal relation to trauma was positively excluded. In cases in which a cerebral injury was assumed to have produced this disease, the analysis proved beyond doubt that only a schizophrenia-like psychosis, and not genuine schizophrenia, was present.

E. L. MILOSLAVICH.

MEDICOLEGAL IMPORTANCE OF THE SEROLOGIC M AND N FACTORS OF LANDSTEINER AND LEVINE. F. SCHIFF, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18:41**, 1931.

The author investigated the new serologic properties of human blood that were discovered several years ago by Landsteiner and Levine, allowing one to differentiate individual bloods besides the ordinary blood groups. These new immune bodies are designated M and N factors of human serum. The author discusses in detail the theoretical principles and the inheritance of the M and N factors, and concludes from an extensive series of experiments that the M and N properties of blood can be demonstrated definitely in new-born infants and in adults; quantitatively, there is no difference between their occurrence in infant and adult blood. The M and N factors are demonstrable even in fetal blood. The inheritance of the factors follows Mendel's laws. The complicated laboratory procedure is discussed in detail. The technic requires the preparation of the immune serums, rabbits being used to obtain the M and N specific serums, the technic of the elective absorption of the immune serums, and finally the diagnosis proper. The laboratory tests are elaborate and should be performed in well equipped institutions only. From four years of his own experimental research, the author concludes that, for identification of blood spots, at the present time the tests are not so completely perfected as to be practically reliable. The serologic identification of the M and N factors may, however, be satisfactorily used in cases of questionable paternity. The author discusses his experience in 537 medicolegal cases and compares the old method, in which use is made of the blood groups, with the new procedure, concluding that the discovery of the M and N factors is a great aid in excluding alleged paternity.

E. L. MILOSLAVICH.

ACTION OF VARIOUS FABRICS ON ISOHEMO-AGGLUTINATION. W. N. ZIPP, *Deutsche Ztschr. f. d. ges. gerichtl. Med.* **18:66**, 1931.

Substances that are commonly used in the preparation of various fabrics, such as in finishing and tanning processes, may influence the serum agglutinins because of an absorbing or destructive action of the chemical present. Certain fabrics that were placed and kept in serum for a period of time for the purpose of absorbing the agglutinins gave out, in the serum, substances that acted destructively on the control erythrocytes. These facts are illustrated by a case in which blood spots found on an overcoat showed the blood group AB, instead of O, which was the correct type. If one undertakes to determine the blood group of blood spots found on fabrics, one should first establish the action of the fabric extracts on the agglutinins.

E. L. MILOSLAVICH.

A CASE OF SUICIDE BY THE USE OF DIMETHYL-SULPHATE. R. BÖRNER, *Frankfurt. Ztschr. f. Path.* **41:367**, 1931.

As a result of the taking of dimethyl-sulphate per mouth, a marked necrotizing inflammation of the base of the tongue, the palate, the esophagus and the stomach was found. There was evidenced a diffuse necrotizing tracheitis and bronchitis and confluent bronchopneumonia. Marked acute lymphadenitis involving the lymph nodes of the neck, the mediastinum and the hilus of the lung was observed. There were also acute splenic hyperplasia, cloudy swelling of the kidneys and marked hyperemia of all organs.

OTTO SAPHIR.

THE PATHOLOGIC-ANATOMIC BASES FOR SUDDEN DEATHS. H. DÜRCK, München. med. Wchnschr. 78:627, 1931.

Dürk records his study of fifty bodies of persons who died suddenly. One death was directly due to trauma, although it had originally been ascribed to heart failure. This occurred in a man who died of an extensive hemorrhage into the pleural cavities and the pericardial sac after a severe injury to the chest by a board hurled at him from a circular saw. Death was almost instantaneous, and bodily injuries were not visible externally. In the remaining forty-nine bodies, death was due to some internal disease. Only a few of these are elaborated on, but a summary of the various groups indicates the range covered. There was one death from pulmonary embolism nine days after an injury to the knee, one from acute cardiac dilatation in a bicycle rider, and one from embolism of the basilar artery, the thrombi originating in the prostatic venous plexus and traversing a patent foramen ovale. Fat embolism of the lungs was found in a stout man's body after he had jumped from a height of 3 meters; he had, however, sustained no marked damage to tissue or fractures of bone. Four deaths were caused by internal hemorrhagic pachymeningitis, and a similar number were attributed to fatty heart, three of those dead from this cause having worked in breweries. Coronary sclerosis was the cause of fourteen deaths and syphilitic aortitis of fifteen. Of the more uncommon causes of death sideroconiosis and a cysticercus cyst of the myocardium were each seen once.

GEORGE RUKSTINAT.

THE RECOGNITION OF POISONING IN CORPSES. K. MEIXNER, München. med. Wchnschr. 78:1750, 1931.

Meixner reiterates most of the well known procedures for detecting poisons in corpses, but stresses a few points that are especially important. He advocates an examination of the brain before other portions of the body, in order to detect odors that might otherwise be obscured. When an odor in the brain is faint, he places portions of the organ in a covered glass container and finds the odor intensified after several hours. He urges the sending of large portions of organs for chemical examination, such as half the liver. He points out the hazards of cutting the brain before it has been hardened in formaldehyde and mentions particularly the softening of the cranial nuclei known to occur in carbon monoxide poisoning.

GEORGE RUKSTINAT.

## Tumors

ADAMANTINOMA OF THE CRANIOPHARYNGEAL DUCT. C. H. FRAZIER and B. J. ALPERS, Arch. Neurol. & Psychiat. 26:905, 1931.

Of 244 cases of sellar and parasellar tumors observed in the neurosurgical clinic of the University of Pennsylvania, 14 were classified by Frazier and Alpers as adamantinomas, also known as tumors of Rathke's pouch, the craniopharyngeal duct and the hypophyseal duct and as craniopharyngiomas. Though they all arise from the same group of cells that give rise to the enamel organ and in the same region of the brain, the authors consider them separate types; that is, adamantinomas and tumors of Rathke's pouch are considered different tumors. For the former, they offer the term ameloblastomas, as ameloblasts (enameloblasts) are the most constant group of cells in them and are essential for the diagnosis. The tumors are generally quite large, extending from the optic chiasm to the anterior border of the pons, are always calcified, and often exhibit cystic degeneration. Aside from the usual signs of tumors of the brain, in seven cases signs of pituitary dysfunction were also present — dwarfism, adiposity, femininity, regressive sex characteristics and other signs.

GEORGE B. HASSIN.

TUMORS OF THE BASAL GANGLIA. FRANÇOIS ODY, Arch. Neurol. & Psychiat. **27:249**, 1932.

The main type of the twenty-five gliomatous tumors found by Ody in the basal ganglions, corpus striatum, thalamus and subthalamus, was multiforme glioblastoma; the less frequent types encountered were astrocytoma and unipolar spongioblastoma. No specific clinical picture could be established from the careful study of these cases. The majority of the symptoms were those of partial decerebration. The latter, however, occurs also in some other conditions, for instance, basal meningitis and intraventricular hemorrhages.

GEORGE B. HASSIN.

PRIMARY FIBROBLASTOMA OF THE BRAIN. B. J. ALPERS, J. C. YASKIN and F. C. GRANT, Arch. Neurol. & Psychiat. **27:270**, 1932.

The tumor, the fourth recorded in the literature, was lobulated, hard and encapsulated. It was 5 cm. long, 2.5 cm. wide and 5 cm. deep, and was situated in the middle of the right motor cortex. The cells of the tumor "ran in streamlets and bands, interdigitating and intertwining like a braid." The intercellular substance contained collagen and some reticulin, but the bulk was made up of fibrous cells and fibrils which apparently were fibroglia. The cells were quite numerous around the blood vessels, and it was suggestive that the origin of the tumor was either in so-called pericytes or in the prolongations of the pia.

GEORGE B. HASSIN.

PAGET'S DISEASE AND OSTEOGENIC SARCOMA. B. L. COLEY and C. S. SHARP, Arch. Surg. **23:918**, 1931.

Three cases of Paget's disease and osteogenic sarcoma are reported, with a review of the literature, and it is suggested that these tumors be considered apart from the general group of osteogenic sarcomas because of their different clinical features. The literature did not reveal a single instance of benign giant cell tumor or endothelioma occurring in bone that was the site of preexisting Paget's disease. This study covers seventy-one cases of osteogenic sarcoma in patients over 50 years of age. Twenty of these were from the records of the Memorial Hospital, New York, and fifty-one from the Bone Sarcoma Registry. The percentage of Paget's disease and osteogenic sarcoma in this series of patients over 50 years of age was 28 per cent. In the series from the Memorial Hospital there were no cases of Paget's disease complicated by osteogenic sarcoma in patients under 50 years of age. In the series of Paget's disease with osteogenic sarcoma, men were affected five times more frequently than women. The bones involved most frequently were the femur, scapula and humerus. One hundred per cent of the cases of osteogenic sarcoma of the skull occurred on the basis of preexisting Paget's disease. Therefore, in a patient over 50 years of age presenting osteogenic sarcoma of the skull there is a strong probability of associated Paget's disease. In this series there was no instance of osteogenic sarcoma involving a portion of the skeleton not involved by the Paget's disease. The histologic picture differs slightly from that of uncomplicated osteogenic sarcoma in that there are present large numbers of giant cells, which are generally hyperchromatic and not necessarily multinuclear. The mortality rate was 100 per cent, and there was no instance of survival beyond a period of five years.

The evidence in this series is to the effect that Paget's disease was present for from ten to fifteen years prior to the development of sarcoma. Comparison of the duration of life in these cases of osteogenic sarcoma in Paget's disease with that in cases of uncomplicated osteogenic sarcoma shows that the duration of life in the former is slightly shorter. The tumor is relatively resistant to irradiation.

N. ENZER.



ADENOMA OF THE HYPOPHYSIS WITH MULTIPLE BONE METASTASES. T. VASILIU, *Virchows Arch. f. path. Anat.* **276**:141, 1930.

The case reported presented multiple tumor metastases to the sternum, ribs, skull and long bones. The nodules were infiltrative. They were white, with yellowish, necrotic centers. Histologically, they were composed of large, epithelioid, eosinophil cells, with very little intercellular substance. The hypophysis appeared normal grossly, but microscopically there was found an adenoma, the structure of which was identical with that of the metastases. In instances of metastasis, when a primary tumor cannot be found, the hypophysis should be examined microscopically.

W. SAPHIR.

POLYPOID SARCOMA OF THE VAGINA. M. DUGGE, *Virchows Arch. f. path. Anat.* **277**:1, 1930.

The author reports a case of polypoid sarcoma of the vagina in an infant 10 months old. The case was unusual in that metastasis to the lung had occurred. Although these tumors are highly malignant, the metastases are usually local, Dugge's case being the first reported with metastases to the lung. The classification of the polypoid sarcomas of the vagina is still unsettled. Since they most often arise early in life and contain tissues of diverse origin, especially striated muscle as in the present instance, they are usually placed among the congenital mixed tumors derived from misplaced undifferentiated embryonal tissues.

W. SAPHIR.

PRIMARY RETOTHELIAL SARCOMA OF LYMPH NODES. F. ROULET, *Virchows Arch. f. path. Anat.* **277**:15, 1930.

The normal lymph node contains four types of differentiated cells of mesenchymal origin. These are the lymphocyte, the fibrocyte of the connective tissue framework, endothelium and the reticulum or retothelial cell. To the three previously recognized types of tumors primary in the lymph nodes, namely, the lymphosarcoma or lymphoblastoma, the fibrosarcoma or fibroblastoma, and the endothelioma or endothelioblastoma, must be added a fourth group only more recently recognized, the reticulum cell or retothelial sarcoma. Tumors of the last-named group are characterized histologically by proliferation of the reticulo-endothelial cells and by the formation of reticulum fibrils. The lymphocytes are compressed and atrophied by the proliferated retothelial tissue and become greatly reduced in number, finally disappearing almost completely. The reticulum fibrils form a dense network, which may undergo collagenous transformation. The capsule is uninvolved in the early stages. Clinically, the tumors are characterized by slow growth and by infrequency of metastasis. In the cases described, with one exception, the growth attacked the lymph nodes of the upper part of the body. The distribution was: cervical nodes, three cases; axillary nodes, two; mediastinal nodes, three, and inguinal nodes, one.

W. SAPHIR.

# Society Transactions

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## CHICAGO PATHOLOGICAL SOCIETY

R. H. JAFFÉ, *President, in the Chair*

*Regular Monthly Meeting, Jan. 11, 1932*

### METASTASIZING LEIOMYOMA OF THE STOMACH. PERRY J. MELNICK.

Gastric myomas are rare. At present 319 have been reported.

A white man, 50 years of age, bled to death from an obscure gastro-intestinal hemorrhage. The postmortem examination revealed an ulcerated leiomyoma of the stomach, composed of mature, fully differentiated smooth muscle cells, without anaplasia or invasion, and a metastasis in the liver which almost exactly resembled the primary tumor.

Benign metastasizing tumors of various kinds have been described, but the descriptions of only a few of these are convincing. Such tumors show a complete discrepancy between their histologic appearance and their malignancy, and are therefore of interest with regard to the attempts at histologic grading of the degree of malignancy of tumors.

There has been much objection to the grading of tumors. Malignancy depends on factors such as compression or obstruction or perforation of vital organs, etc., as well as on characteristics of growth as revealed by the histologic structure. Also, the histologic criteria involved have not been proved. Furthermore, the resistance or immunity of the patient has not been considered. Although two tumor cells may appear identical in different persons even in different organs, they will behave differently.

Cells of benign tumors may enter blood vessels, but are probably immediately destroyed. However, if in their new environment they should find favorable circumstances, they may continue to grow. This conception of the resistance or of the status of the individual is important in considering the etiology of tumors.

### A STUDY OF PHOSPHORUS PARTITION IN EXPERIMENTAL DEGENERATION OF STRIATED MUSCLE. D. K. FISBACK and H. R. FISBACK.

Acute molecular degeneration of striated muscle was produced in rabbits by a standard method of contusion, and the degenerated muscle was studied chemically within forty-eight hours. Fiske and Subbartow's methods for the determination of phosphocreatine and inorganic phosphorus were used.

In the control animals, the phosphocreatine values were from 61 to 72 mg. per cent, with an average of 68 mg. In degenerated muscles, this average was decreased to 7 mg. There was but little change produced in inorganic phosphorus in degenerated muscles. The control range of 33 to 37 mg. per hundred cubic centimeters, with an average of 34 mg., was altered in the abnormal muscles to a range of from 32 to 45 mg., with an average of 37 mg.

### NEUROBLASTOMA OF THE SUPRARENAL GLAND, WITH MULTIPLE METASTASES. JACOB KLEIN.

A girl, aged 2½ years, ill for seven weeks, had a firm, smooth mass the size of a hickory nut at the angle of the right lower jaw, marked pallor, abdominal pains, marked night sweats and difficulty in walking. The abdomen was markedly distended; the liver was 4 fingerbreadths below the costal margin; the superficial veins on the abdomen and thorax were distended. The blood had a hemoglobin content of 45 per cent; the erythrocyte count was 1,250,000; the leukocyte count

was 8,000; the differential count was: polymorphonuclear leukocytes 31 per cent, lymphocytes 58 per cent, monocytes 5 per cent and myelocytes 6 per cent. The Wassermann reaction of the blood was negative. Roentgen examination demonstrated a pathologic fracture of the right humerus just below the upper epiphysis. There was marked erosion of the left humerus in the same region. Practically all of the long bones showed periosteal proliferation.

The child died after a further illness of seven weeks. The liver was markedly enlarged and had many small, red-blue masses. The spleen also was slightly enlarged, and the surface had several similar red regions. The right suprarenal gland was replaced by an encapsulated dark red, soft tumor, which compressed the kidney and the right lobe of the liver. The calvarium, the long bones, the postorbital spaces, the abdominal lymph nodes and the osteochondral junctions of the ribs had tumor metastases.

Microscopically, the tumor consisted of small, round cells with dark-staining nuclei. The cells were arranged in groups, some necrotic. The nuclei surrounding these necrotic regions assumed the form of pseudorosets. Hortega's and Mallory's glia stains showed glia fibers throughout the tumor. The liver, lungs, spleen and lymph nodes had similar tumor cells. The diagnosis was primary sympathicoblastoma of the medulla of the right suprarenal gland with metastases to the lymph nodes, calvarium, left humerus, ribs, orbital fat tissues, liver, lungs and spleen, and pathologic fracture of the right humerus.

#### HEMORRHAGE INTO THE STROMA OF BOTH OVARIES. A SEQUEL OF MITRAL STENOSIS. GEORGE RUKSTINAT.

The ovaries were obtained from the body of an unmarried woman, aged 38, who died of cardiac decompensation. Following scarlet fever at the age of 12, she was an invalid with marked dyspnea and edema of the lower extremities, and repeatedly had had such marked cardiac decompensation that death seemed imminent. When admitted to the Frances E. Willard Hospital she had labored, shallow respirations, about 40 per minute; a pulse only occasionally perceptible, and a systolic murmur audible 6 inches (15 cm.) away from the chest. She lived in the hospital fifty-seven hours. Her temperature was 97.2 F. rectally until six hours before death, when it rose to 99 F.

The essential items of the anatomic diagnosis were: chronic, indurative, deforming and acute thrombo-ulcerative mitral endocarditis; marked mitral stenosis; marked eccentric cardiac hypertrophy; ecchymoses of the pleura, and extensive interstitial hemorrhage of the ovaries. The ovaries were twice the usual size, and were mottled, with subserous hemorrhages up to 5 mm. in diameter, which alternated with a tense, gray-yellow cortex, and dripped with blood when sectioned. Both ovaries were alike; on many surfaces made by cutting, each had a cortex varying in thickness from 1.5 mm. to the thickness of the serosa in the regions of greatest hemorrhage. The medulla resembled a bloody sponge with scattered fibrous septums.

In both ovaries, histologically, the prominent feature was the extensive, recent hemorrhage. The red blood corpuscles were well preserved, and all vessels, arteries, veins and capillaries were hugely distended. In all sections, the walls of the arteries were intact, although frequently they were entirely surrounded by blood cells, which penetrated the stroma. Follicles in any stage of development were few, but corpora albicantia were numerous, often with multiple peripheral bloody impregnations continuous with the interstitial extravasations. In many regions nothing but blood was discernible in the central portions of the ovary, but toward the germinal regions columns of blood cells, 1 cell wide, alternated with a single layer of spindle-shaped cells from the shredded stroma. The bleeding in both ovaries was from the capillaries and smaller veins, in many of which actual rupture of the vessel was demonstrable. Bleeding from many places in the medullary part of each ovary had converted them into oval masses with bloody projections, so that they resembled mulberries. These excrescences were seen easily through the serosa. In the absence of inflammatory changes and with

profound pelvic hyperemia, the ovarian hemorrhages probably were due to stasis. This stasis, in the pelvis, was accentuated during life by the semi-Fowler's posture and by the increased intra-abdominal pressure.

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*Regular Monthly Meeting, Feb. 8, 1932*

R. H. JAFFÉ, *President, in the Chair*

ANATOMIC CHANGES IN THE LIVERS OF DOGS FOLLOWING MECHANICAL CON-  
STRICTION OF THE HEPATIC VEINS. J. P. SIMONDS and J. W. CALLAWAY.

The livers of dogs examined twenty-four, forty-eight and seventy-two hours and seven days after mechanical obstruction of the hepatic veins for from seven to thirty minutes showed the following changes: A mean increase of 25 per cent in the ratio of liver weight to body weight, due to edema and to swelling of the hepatic cells; swelling, granulation, vacuolization and extensive necrosis of the hepatic cells in the central half or two thirds of the hepatic lobules; marked dilatation of the perivascular lymphatics surrounding the sublobular veins; the presence of hyaline thrombi in many central and sublobular veins; intrasinusoidal cell masses of two types: (a) small, compact, occluding masses, probably originating in "conglutination thrombi" of red cells, and (b) larger, more diffuse, branching cell masses; hemosiderosis of Kupffer's cells.

The report in full will be published in the *American Journal of Pathology*.

LUMBOSACRAL TERATOMA ASSOCIATED WITH SPINA BIFIDA OCCULTA. PAUL C.  
BUCY and H. E. HAYMOND.

In contrast to sacral and sacrococcygeal teratomas, similar lumbosacral growths are exceedingly rare.

A girl, 15 months of age, had had a lump present over the lower part of the back since birth. The mental and physical development had been normal. Roentgen examination revealed a marked defect of the posterior portions of the neural arches of the fourth and fifth lumbar vertebrae and of the entire sacrum. Laboratory examination showed an unexplained leukocytosis with counts varying from 11,850 to 27,000.

Under ether anesthesia, the lumbosacral mass, measuring 10.5 by 9 by 4 cm., was removed. It was found to be attached by a pedicle through the defect of the posterior vertebral arch. The postoperative course was uneventful.

The tumor was cystic, and the cyst was filled with mucinous fluid. The stained fluid contained cells in all stages of transition from lymphocytes to macrophages. The cyst wall was lined by cells resembling those of respiratory epithelium. Other sections of the tumor revealed loose connective tissue, many bundles of myelinated nerve fibers, a ganglion containing many ganglion cells, an atypical pacinian corpuscle, a lymph node and much smooth muscle. No cartilage or bone was present.

The reports by Sonntag (1925) and Aloï (1931) are the only others since 1800.

A PRIMARY PULMONARY TUBERCLE APPEARING IN A PATIENT HAVING  
ADVANCED HODGKIN'S DISEASE. H. C. SWEANY.

This paper is to be published in the ARCHIVES.

THE INFLUENCE OF THE REACTION OF URINE ON THE GROWTH OF BACTERIA.  
RUSSELL D. HERROLD and EARL E. EWERT.

Others have made observations on the hydrogen ion concentration of urine and its relation to infections, and have noted that the reaction of urine may be secondary to the growth of bacteria in vivo, particularly in residual urine. We have

studied both clear and contaminated urine. Comparison has been made of the hydrogen ion concentration and cultural results of fresh specimens with those of the same urine after incubation for intervals of from a few hours to several days.

The usual reaction of urine is acid. We have also made hydrogen ion readings of specimens in a series of one hundred chronic infections of the urethra and its adnexa with macroscopically clear urine. The results may be grouped as follows:  $p_{\text{H}}$  4.8 to 5.2 in twenty-nine; 5.3 to 5.7 in thirty; 5.8 to 6.2 in eighteen; 6.3 to 6.7 in thirteen; 6.8 to 7.2 in ten. In a series of twenty active infections with colon bacilli, the reaction did not occur with greater frequency in any one of the groups of  $p_{\text{H}}$  readings. These urines contained sufficient exudate and bacteria to be hazy or cloudy. They were tested immediately after collection.

White and Winter stated that the average reaction in the urine of patients with active gonococcus infection is about  $p_{\text{H}}$  6.8. Our results indicate a higher acidity in these infections, so that they parallel the diversified range of uninfected urines. Likewise we have found that the reaction of the urine is not influenced by the presence of tuberculosis of the genito-urinary tract, or in colon bacillus infections except in the presence of marked residual urine, and here the influence is less than when a staphylococcus is the infectious agent under the same condition.

The rapid growth of staphylococci in urine coincident with a change of the hydrogen ion concentration toward the alkaline side within six hours seems to indicate that the reaction of the urine may be influenced by such infections even without residual urine. The change toward the alkaline side with the growth of staphylococci or other gram-positive micrococci in vitro explains the strong ammoniacal or alkaline urine when residual urine is infected with these organisms. The rapidity of change toward the alkaline side is in direct proportion to the hydrogen ion concentration at the time of inoculation, so that the stronger the acidity the greater the lag period before alkalinity is reached by the growth of the bacteria. This observation is equally true of urine that has been inoculated with colon bacilli.

In smears bacteria may be seen that do not grow by the usual cultural method. In several catheterized urines that failed to give growth when inoculated in the fresh state on solid mediums, bacteria developed after incubation; on subcultures after intervals of from twenty-four to seventy-two hours staphylococci were isolated. Streptococci have appeared in association with the staphylococci in some instances. This suggests that an inhibiting substance is transferred to the solid medium from fresh specimens which prevents growth at this time, but which may be inactivated by incubation of the urine. Infection accompanied with a crystal clear urine and sterile cultures on the usual solid medium is associated with disturbance of the bladder, and this entity has frequently been classified as cystalgia. It also might explain the sterile cultures in those conditions that have been named solitary or elusive ulcers of the bladder. The presence of bacteria in the stained preparation in many instances seems to eliminate accidental contamination as does also the repetition of the same cultural results in specimens taken at repeated intervals. During the treatment of active infections by means of acidifying agents with or without urinary antiseptics, frequently colon bacilli do not grow in the subcultures of the fresh specimens, whereas subcultures after incubation for from twenty-four to forty-eight hours yield large numbers of colon bacilli. As a criterion of cure, therefore, subcultures of such specimens after incubation must remain sterile.

An incubation of bacteria for from three to ten days in the alkaline range above  $p_{\text{H}}$  8.6 is bactericidal as a rule for organisms of the genito-urinary tract with the exception of *Bacillus pyocyaneus* and a small group of colon bacilli. Such incubation in the presence of acidity greater than  $p_{\text{H}}$  5.2 has a like effect. Our observations so far indicate that it is more difficult to obtain a consistent high alkaline range than a high acid range for purposes of treatment. Therefore, we have followed a number of colon bacillus infections by means of acidifying agents, and so far have found that, of several drugs tried, ammonium chloride (enteric coated) is best for this purpose, and is well tolerated in divided doses of 90 grains (5.8 Gm.) daily. In many instances, bacteriologic cure has been obtained

with acidifying drugs as promptly when these are used alone as when they are combined with hexamethylenamine. A comparison was made of gonococcal tests on a single urine made more acid or more alkaline by the addition of acid sodium phosphate or alkaline sodium phosphate. Five specimens were so tested, each with the following  $p_{\text{H}}$  values: 4.8, 5.3, 6.2, 7.1 and 7.6. Gonococci were added to each of these urines, and subcultures were made after twenty minutes. No growth was obtained in the mixture of urine and gonococci of  $p_{\text{H}}$  4.8, but some growth was obtained in all the others. Additional subcultures were made after twenty-four hours, and growth was obtained only in the specimen of  $p_{\text{H}}$  7.1. Of the aforementioned  $p_{\text{H}}$  values, 4.8 is quickly bactericidal for the gonococcus, and all but 7.1 are bactericidal with longer periods of contact.

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## NEW YORK PATHOLOGICAL SOCIETY

*Regular Meeting, Jan. 28, 1932*

### REGENERATION OF RED CELLS FOLLOWING COPPER AND IRON THERAPY. B. R. WHITCHER.

By recent investigations on rats, copper in combination with iron has been found to increase the regeneration of hemoglobin and red cells.

During the past year approximately one hundred infants and young children from the Children's Clinic of the New York Post-Graduate Hospital have been treated with a combination of soluble salts of copper and iron. The period of treatment varied from four to fourteen weeks. In the large majority of cases, the red cells rose from an initial count ranging from 3,200,000 to 3,900,000 with from 52 to 75 per cent hemoglobin, to between 4,300,000 and 5,150,000 with from 82 to 94 per cent hemoglobin. With the improvement in the blood picture there was a similar improvement in the appetite, the general physical condition and the appearance of the skin and mucous membranes of the patient.

A few of the children suffered from intercurrent gastro-intestinal disturbance or respiratory infection during the course of treatment, and a slight fall occurred in the red cell count and hemoglobin percentage, which rose after the infectious process had subsided.

The study of these cases indicates the beneficial effects of copper in combination with iron on the regeneration of the red cells and hemoglobin in the secondary anemias of young children. Its usefulness is impaired when a severe digestive or respiratory disturbance or other infectious process occurs during the course of treatment.

### A CASE OF RENAL BLASTOMA WITH CRANIAL METASTASIS. J. S. GREWAL.

A white boy, 11 months of age, was admitted to the New York Post-Graduate Hospital on April 7, 1931, with the following history: A full-term child, delivered normally and weighing 9 pounds 8 ounces (4,309 Gm.), he had been apparently well until one month before admission. At that time slight cough and coryza developed, without fever. Thereafter the child took his food poorly and became progressively pale. About two weeks later, it was noticed that he held his head toward the right side, and that the mouth was pulled to the left, especially on crying. It was also noticed that the right eye remained open when the patient slept. About one week before admission, a black and blue discoloration was noticed over the lids of the right eye.

Physical examination revealed a markedly anemic white child, about 11 months of age. The skull had irregular prominent bosses. The anterior fontanel was about 1 cm. in diameter. There was dusky discoloration of the lids of the right eye. The right pupil was greater than the left. The right palpebral fissure was greater than the left. The right pupil did not react to light. Consensual reflex was present from right to left, but absent from left to right. The wink reflex was absent in the right eye. The optic disks were pale. The retina revealed minute

spots, simulating congenital chorioretinitis in the right eye. There was drooping of a corner of the mouth. Marked asymmetry of the face was noticed as the child cried, the mouth being drawn to the left. The abdomen was markedly protuberant. There was a large, hard, irregular mass occupying the right half of the abdominal cavity, extending from the crest of the right ileum to the costal border on that side. The genitalia showed slight edema of the scrotum with some purpuric discoloration. Superficial and deep reflexes were active and equal. Rectal examination gave negative results.

Urinalysis showed nothing significant. Examination of the blood showed: red blood cells, 1,630,000; white blood cells, 12,000; hemoglobin, 24 per cent; platelets, 108,000; bleeding time, six minutes; clotting time, four minutes.

On April 8, about 275 cc. of blood was given intravenously by the Unger method. The child refused food with the exception of milk. He had been increasingly thirsty during the last few days. He passed a number of tarry stools during the last day. Death occurred on April 10, 1931.

Autopsy was performed on April 10, 1931. The general description of the body post mortem is similar to that given in a foregoing paragraph. When the brain was removed, the base of the skull showed an invasion by tumor in the following regions: (1) the cribriform plate, especially the right side; (2) the right orbital fissure, where the growth had destroyed the greater and lesser wing of the sphenoid; (3) the right wall of the sella turcica, with extension into the right cavernous sinus; (4) extension into the right petrous sinus; (5) the left middle fossa, and (6) the left (occipital) posterior fossa. Along these areas the dura was raised to a height of about from 5 to 8 mm. The tissue was soft, hemorrhagic and dark reddish purple. The bones were eroded.

In the thorax, the right third rib (at its angle) and the mediastinal lymph nodes showed invasion by soft, hemorrhagic tumor tissue. In the abdomen, the ascending colon was pushed to the left up to the midline by a large, nodular, hemorrhagic mass, which occupied the entire right half of the abdominal cavity. The cecum with a portion of ascending colon was adherent to the anterior surface of the mass. The serosa showed marked congestion and numerous petechial hemorrhages. The tumor mass appeared to be arising from the right kidney, only a small posterolateral portion of which could at the time be recognized. The upper pole was firmly adherent to the under surface of the right lobe of the liver. The right suprarenal gland was of normal size and located at its upper pole but more medially and anteriorly toward the midline. The medial portion of the tumor completely encircled the aorta and the inferior vena cava and at one point invaded the head of the pancreas. The surface of this tumor mass was nodular but completely encapsulated, so that it was removed en masse by cutting across the upper and lower ends of the abdominal portion of the aorta. The upper pole, however, was firmly adherent to the liver. The entire tumor mass measured 150 by 100 by 80 mm. Numerous enlarged, hemorrhagic, soft mesenteric lymph nodes are also present. On tracing the right ureter down from the pelvis and up from the bladder, at a point 2 cm. above its distal end, a complete stricture was encountered. The ureter above this point was slightly distended by turbid, blood-tinged fluid. The pelvis and calices, which were only poorly preserved, did not show any dilatation.

Histologically, the tumor tissue consisted of nests of cells, which did not seem to show any tendency toward differentiation. The individual cell was made up of a vesicular nucleus and, around this, a small amount of clear, pale blue-staining cytoplasm, the outer border of which was not well defined. These cells were present in large clusters held by a very small amount of edematous fibrous tissue. Extensive interstitial hemorrhages were present throughout the tumor tissue. At the point of transition from normal-appearing renal tissue to the tumor mass, small cords and rounded clusters of tumor cells were found lying alongside of fully formed tubules and glomeruli. Here some abortive tubules and glomeruli could also be recognized.

The tumor undoubtedly arose from embryonal cells, which were intended to differentiate into metanephric renal tubules. A portion of such a kidney was

undoubtedly formed, but most of these cells had failed to differentiate and continued to multiply in an irregular, lawless fashion.

THE RELATION OF THE TONSIL TO BRANCHIOGENETIC CYSTS. LOUISE H. MEEKER.

Many of the cysts of the neck, and in particular those near the angle of the jaw, are tonsillar in origin. They are characterized by an abundance of true lymphoid tissue in the walls.

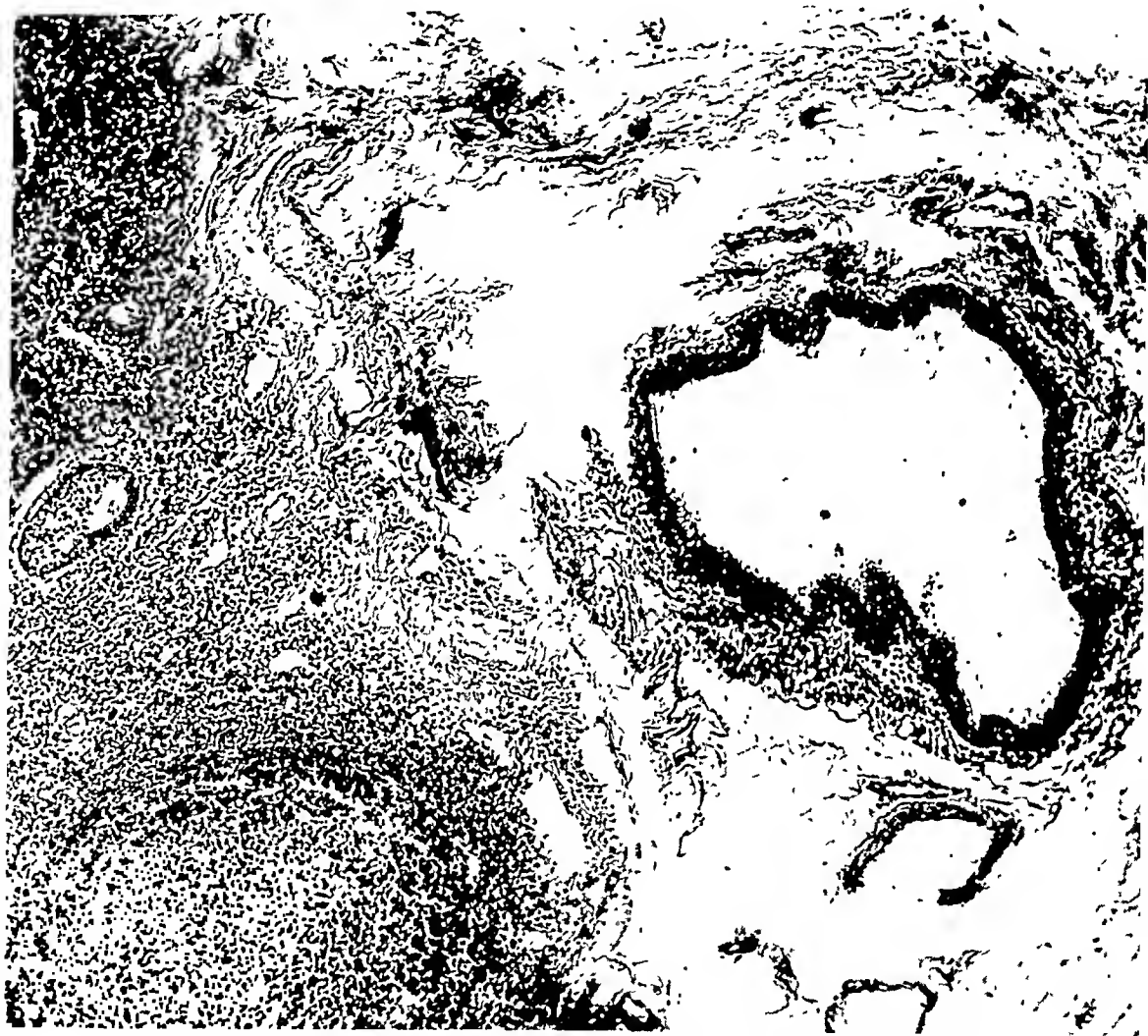


Fig. 1 (M. 28).—A fistula beneath a tonsil. It is lined by ciliated columnar epithelium. Above, at the center, the fundus of an associated crypt is lined in part by ciliated columnar epithelium. The fistula led to the angle of the jaw.

The first observation in regard to these cysts was made by Lucke, in 1861. His diagnosis was that of an epithelial rest in a cervical lymph node, the fetal rest of Cohnheim.

Darrier, in 1894, saw a fistula with a similar lining and was the first to consider the relation of these cysts to the pharynx.

It remained for Terrier and Lecene, in 1905, to collect the reports of cysts of this type and to add two of their own. They suggested the term "type



amygdaline." Cotelloni agreed that they were quite distinct from the dermoid cysts, with which they had previously been classed because of their lining of squamous epithelium.

Albrecht reported cysts having true lymphoid tissue in the walls, but lined by cylindric epithelium. Cysts of this type with papillary formations have been



Fig. 2 (M. 40).—A cyst beneath a tonsil. It is lined by ciliated columnar epithelium. The patient had a history of peritonsillar abscess for many years, and had recently had an abscess of the neck.

reported by Peyron, Houdart, Hulnagal and Warthin. Peyron and Rouislacoix found bodies resembling Hassall's corpuscles in cysts with walls of lymphoid tissue. These they designated "types Hassallienes."

I am attempting to show that any of these types may arise in the tonsillar region. According to Kingsbury, the upper lobe of the tonsil develops in the

second pharyngeal pouch and the lower lobe from an adjacent area in the third pharyngeal pouch closely related to the indefinite branchiomeres from which the thymus may or may not develop.

In the study of the relation of mucous glands to the tonsil, I demonstrated columnar epithelium, both with and without cilia, in the fundi of crypts. In some instances these were in areas in which ducts of the mucous glands opened into the crypts. In other instances there were no related mucous glands. Instead I was able to demonstrate associated minor branchiogenic clefts.

The French chiefly have written on this subject, as in the collected cases of Thomann, 1925, and Moatti, 1929; Moatti's series included three examples of "cystes amygdalines," which he said resembled both tonsil and thymus.

Cysts with walls of true lymphoid tissue lined in part by columnar epithelium and in part by squamous epithelium or by ciliated columnar epithelium may also be considered as of tonsillar origin, in some instances at least.

THE INFLUENCE OF BLOOD AND OF EXUDATE ON THE ACTION OF BACTERIOPHAGE AGAINST THE COLON BACILLUS. MARTHA APPLEBAUM (by invitation) and WARD J. MACNEAL.

In a previous paper (Applebaum, Martha, and MacNeal, Ward J.: *J. Infect. Dis.* 49:225, 1931), we reported experiments on the influence of pus and blood on bacteriophage, particularly that of the staphylococcus. It was found that these body fluids exerted an inhibitory effect on the action of the staphylococcus phage, but results for the colon bacillus were inconclusive; for the strains of this microbe employed failed to grow in the broth to which considerable amounts of blood or exudate had been added.

On further study it has been found that old laboratory strains of the colon bacillus fail to grow in blood, while those recently isolated from the body grow well. With fresh strains, an inhibition of the colon bacillus phage was revealed similar to but not as great as that of the staphylococcus phage. Experiments in which urine was employed in place of broth revealed inhibition of bacteriophage by blood and exudate similar to that found in broth. These findings offer a logical explanation of the unfavorable effect of instrumentation during bacteriophage therapy of the urinary tract. (This work was supported by a grant from the Josiah Macy, Jr., Foundation.)

The paper will appear in full in the *Journal of Infectious Diseases*.

COMMERICAL BACTERIOPHAGE PRODUCTS. MARTHA APPLEBAUM (by invitation) and MARGARET E. STRAUB (by invitation).

Three companies in this country produce bacteriophage for the medical profession. One company markets a jelly for staphylococcus infections and has fluid preparations for clinical trial for the colon bacillus, the hemolytic streptococcus, the green-producing streptococcus and the indifferent streptococcus. All of these products were tested in vitro against our laboratory stock strains. Our results indicate the presence of an antiseptic, which is now admitted by the manufacturer. The jelly for staphylococcus infections contains, in addition, a weak staphylococcus bacteriophage, probably attenuated by the antiseptic. The streptococcus filtrates exert an inhibitory action against several bacterial species owing to the presence of the antiseptic. This result was evidently not due to a phage, as the inhibitory effect was not transmissible. Our tests failed to discover any bacteriophage in this preparation, as it failed to produce lysis in any of the four strains of streptococci with which it was tested. A streptococcus jel and a colon bacillus jel newly released for medical use will be tested later, and the results will be included in a final paper.

A second manufacturer produces a staphylococcus phage in fluid form, which contains no antiseptic, but does not contain a sufficiently potent phage.

A third manufacturer has a potent staphylococcus phage in fluid form without the addition of a preservative. This manufacturer's staphylococcus phage-colon

phage mixture contains no recognizable staphylococcus phage, while the colon bacillus phage is weak in potency.

Bacteriophage therapy is still on trial. Perhaps it is too early to expect reliability in commercial products of this nature. Physicians who employ such products are advised to have them tested in a reliable laboratory for potency against the infectious microbe of the particular patient under treatment. (This work was supported in part by a grant from the Josiah Macy, Jr., Foundation.)

BACTERIOPHAGE AS A THERAPEUTIC AGENT IN STAPHYLOCOCCUS BACTERIA.  
WARD J. MACNEAL and FRANCES C. FRISBEE (by invitation).

The staphylococcus phage has been employed in various ways in the treatment of fifteen patients suffering from staphylococcus infection of the blood stream. Eight of these were observed by the authors at the New York Post-Graduate Hospital; four were seen by one of us outside the hospital, and three others were treated by other physicians in cooperation with us but without the patients being seen by us.

Two preparations of a pooled mixture of bacteriophages were used, one prepared in nutrient broth for local applications and the other prepared in an almost protein-free asparagine medium for intravenous injection.

Of the three patients not seen by us, one had a fulminating staphylococcus septicemia of cryptic origin and died on the fourth day of his illness after receiving only 0.75 cc. of the asparagine bacteriophage in two intravenous doses on the day of death; the other two recovered after prolonged intravenous treatment coupled with local application of bacteriophage to the carbuncle on the face and the cellulitis of the foot in the respective patients.

The four patients seen in consultation outside the hospital were regarded as moribund when the use of bacteriophage was begun. One of these, a woman with furuncle of the face, extensive cellulitis and repeated positive blood cultures even after jugular ligation, received bacteriophage intravenously and also by multiple punctures around the swollen area on the face; she eventually recovered. Another patient with very similar lesions, starting on the face, received intravenous bacteriophage when first seen by us without local injection until three days later. He died the next day. Two other patients with overwhelming infection of the blood stream when first seen, survived two days and three days, respectively.

Of the eight patients studied in detail in our own hospital, four survived. The earliest one of these made a satisfactory recovery after an illness of many months, during which nephrectomy was performed for an enormous renal abscess. In this case bacteriophage was administered over a long period. The second patient was a baby with multiple abscesses of the scalp, general sepsis and purulent pericarditis, terminating in death. The third patient was a surgeon in whom removal of a ureteral calculus was followed by infection of the blood stream. Large amounts of bacteriophage were used in the wound during four days. Convalescence was prolonged, but the patient is again active in his profession after more than a year of inactivity. The fourth and sixth patients were school boys of 15 and 13 years, respectively, with acute osteomyelitis of the extremities and thoracic complications. Both died, one promptly and the other after receiving over 900 cc. of bacteriophage preparation intravenously in the course of a month. The fifth patient in this series was an old man in whom extensive decubitus and septicemia developed after being in bed for a month subsequent to prostatectomy. A single intravenous injection of 4.5 cc. of asparagine bacteriophage was followed by a chill of forty minutes' duration. Further bacteriophage treatment was thought, at that time, to be contraindicated, and the patient died three days later. The seventh patient was a woman with alarming acute purulent arthritis of the right knee and abundant bacteria in the blood stream. Local and intravenous treatment with bacteriophage was persisted in for weeks, and the patient made a very satisfactory recovery. The eighth patient in this series is a man now convalescent after septic phlebitis and infection of the blood stream following treatment of varicose veins of the leg by injection.

Our ideas have gradually changed during the experience of the last two years. At present, we begin immediately with intravenous injections in divided doses at intervals of about thirty minutes until definite evidence of a shock is obtained, ordinarily a chill with a sharp rise in temperature followed by a fall to nearly normal in twelve hours. At the same time, the bacteriophage is applied to the open wound and injected into the tissues about the local lesion, if any such lesion is in evidence. We also insist that the intravenous injections of smaller amounts shall be continued daily for a long time after the initial shock. The treatment still leaves much to be desired, but already, we think, the outlook for the patient with staphylococci in the blood stream has been considerably improved by the advent of bacteriophage therapy. (This work was supported by a grant from the Josiah Macy, Jr., Foundation.)

#### DISCUSSION

SIMON L. RUSKIN: I would like to ask Dr. MacNeal whether he does not think that the chill that occurs after the giving of bacteriophage therapy may not be due to the increased stimulation of bacterial growth at the onset of bacteriophage therapy, as described by d'Herelle. He feels that at the beginning there is an increase in bacterial growth which, on continuation of the phage, is followed by lysis of the bacteria.

WARD J. MACNEAL: I do not think one can give a satisfactory or certain answer to that question. However, I will say that when small doses are given, for example, 0.25 cc. of a 1:10 dilution, which may be regarded as a stimulative dose, we do not observe a temperature change. Usually after we get the dose up to a good-sized one, the patient gets the shock. We then stop further administration of the bacteriophage, and the patient appears to be better during the next two days. I am rather inclined to think this is a protein shock, or a chemical shock, due to the disintegration of the staphylococci. By injecting a large dose of bacteriophage into a person who has no bacteremia, or into a normal rabbit, one does not get any shock. But if you give it to a person who has an infection of the blood stream, after you reach a moderate dose, then you get shock. I think it is due to disintegration of the bacteria, but I cannot prove it.

## Book Reviews

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**Pathologie und Klinik in Einzeldarstellungen.** Herausgegeben von L. Aschoff, H. Elias, H. Eppinger, C. Sternberg und K. F. Wenckebach. Band IV: Thrombose; ihre Grundlagen und ihre Bedeutung. Von Professor Dr. A. Dietrich, Direktor des Pathologischen Instituts der Universität Tübingen. Paper. Price, 8.80 marks. Pp. 102, with 26 illustrations. Berlin: Julius Springer, 1932.

The monograph is an elaboration of the author's work published in 1920 on "Thrombosis After War Wounds." Experimental data are offered to support the author's concept that infection plays the greatest rôle in thrombosis. The formation of a thrombus depends on a direct reaction between the blood and the endothelium of the vessels. There results thereby a thin fibrinous membrane covering the endothelium which is the foundation of the clot and on which platelets and blood cells are deposited (Klemensiewicz). The latter elements bear no relation to the coagulability.

The mechanism by which the fibrinous membrane forms is explained by a sensitization of the wall of the vessel; the factor of stasis exaggerates the condition and localizes the thrombus. Experimentally, small intimal fibrinous nodules were produced in rabbits by the use of a specific vaccine and organism (*B. coli*). Actual thrombi formed only after sensitization of the organism with a nonspecific protein (casein) and then the injection of a bacterial vaccine (staphylococcus). Because of the low percentage of thrombi produced and because the type of thrombi was coagulative in structure, the experimental evidence in favor of the rôle of vascular sensitization is not convincing.

Clinically chronic infections serve to sensitize the body and thus predispose to thrombosis. The percentage of thrombosis following infection, however, is given as only 19; following cardiovascular diseases plus infection, as 46; following post-operative procedures plus infection, as 92 (Tübingen) and as 47 (Köln). Although it is admitted that infection influences thrombosis, it will always be difficult to determine whether the thrombus formed before or after the infection (Lubarsch). Furthermore, thrombosis and embolism occur most frequently between the fifth and the eleventh day after operation and thus cannot be considered as following chronic infections.

Fatal pulmonary embolism may come about in one of two ways: by numerous small clots blocking over two thirds of the vascular bed or by large clots occluding the main pulmonary artery. The right side of the heart is the most common origin for the small emboli, whereas the femoral veins are the most common origin for the larger emboli. In 53.1 per cent of all thrombi, pulmonary emboli result, 35 per cent being fatal. These values are somewhat higher than those usually reported (for fatal pulmonary embolism, the reported data vary from 1.5 to 25 per cent).

Regarding the increase of thrombosis and embolism, the author is skeptical. Only postmortem material of similar clinics in the same locality can be compared. The numerous studies abiding by these dictums are not given proper recognition.

**The Thomsen Hemagglutination Phenomenon: Production of a Specific Receptor Quality in Red Corpuscles by Bacterial Activity.** By V. Friedenreich. Pp. 137, with 12 figures. Copenhagen: Levin & Munksgaard. 1930.

Oluf Thomsen reported in 1927 (*Ztschr. f. Immunitätsforsch. u. exper. Therap.* vol. 52, p. 85) an artificially produced panagglutinability of human red blood corpuscles as a result of a transmissible agent. Each of the four groups could

be so modified. Agglutination took place also in serums of group AB (no agglutinins) and even in the serum of the person from whom the corpuscles were taken. The agglutination was attributed to a third agglutinin present in any human serum and independent of the iso-agglutinins  $\alpha$  and  $\beta$ . Thomsen attributed the change in the corpuscles to the appearance or activation of a receptor quality designated as "L," normally latent.

The present abstracted publication of Friedenreich is an admirably thorough extension of the investigations of Thomsen. A number of bacteria were isolated which are able to produce the transforming principle. The first of them, in chronological order, was named the "M" bacillus. The second was named the "J" bacillus and was the more active transformer of the two. Both were studied in detail and classified among the coryniform bacteria. Transformation power was also found in some strains of the *Vibrio cholerae* group and in two other bacterial species. The transforming principle is a filtrable product of bacterial metabolism. Its action is in the nature of an enzyme, as it is not used up at the end of the transformation process. The change in the red blood corpuscles is in the nature of a new receptor "T," which reacts with the agglutinin "T," present in all serums but in varying strength. The transformation begins after a short incubation period and progresses to a maximum point. Red blood corpuscles of a number of animal species were found transformable, and the resulting receptor "T" was apparently identical with the human receptor. A specific hemolysis was also observed, but only with blood corpuscles of guinea-pigs, the hemolysin being of a complex nature.

The possible errors in blood grouping resulting from bacterial contamination are pointed out: (a) agglutination of blood corpuscles with both agglutinins, simulating group AB; (b) agglutination of corpuscles originally belonging to group "O" with only one of the two agglutinins, if the agglutinin "T" in one of the two agglutinating serums is too weak, then simulating group A or B. The source of error can be eliminated by performing the grouping not later than from twelve to twenty-four hours after the blood was drawn, before the end of the incubation period, and if that is not possible, by adding from 0.1 to 1 per cent of formaldehyde (40 per cent) to the corpuscular suspension. An additional precaution, particularly in scientific investigations, would consist in testing the serum as well as the corpuscles and in testing the corpuscles with serum AB (no agglutinins), if it is rich in agglutinin "T," and with donor's own serum.

The necessity of using the aforementioned precautions is best illustrated by the fact that in a series of 536 specimens of blood, which were 4 days old when tested by Friedenreich, 30 showed abnormal agglutination, and 18 of those were due to bacterial action. The discoveries of Thomsen and Friedenreich throw a great deal of doubt on the correctness of some results of investigations of blood groups but at the same time offer a simple means of eliminating one of the most tricky sources of error in this field.

## Books Received

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INTELLIGENCE AND DISEASE. By Shepherd Dawson, assisted by J. C. M. Conn. Medical Research Council Special Report Series, No. 162. Price, 1 shilling, net. Pp. 53. London: His Majesty's Stationery Office, 1931.

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF VISION: IX. PSYCHOLOGICAL FACTORS IN PERIPHERAL VISION. By G. G. Grindley. Medical Research Council Special Report Series, No. 163. Price, 1 shilling, net. Pp. 49. London: His Majesty's Stationery Office, 1931.

RECENT ADVANCES IN BACTERIOLOGY AND THE STUDY OF THE INFECTIONS. By J. Henry Dible M.B. (Glas.), F.R.C.P., Professor of Pathology in the University of Liverpool; Late Professor of Pathology in the University of London, and Professor of Pathology and Bacteriology in the Welsh National School of Medicine. Ed. 2. Price, \$3.50. Pp. 476, with 29 illustrations. Philadelphia: P. Blakiston's Son & Co., 1932.

A DOCTOR OF THE 1870's AND 80's. By William Allen Pusey, Sometime President of the American Medical Association and of the American Dermatological Association. Price, \$3.00, postpaid. Pp. 153. Springfield: Charles C. Thomas, 1932.

TUMORS OF BONE. By Charles F. Geschickter, M.D., Surgical Pathological Laboratory, Department of Surgery, Johns Hopkins Hospital and University, and Murray M. Copeland, M.D., Memorial Hospital, New York. With forewords by Dean Lewis, M.D., Professor of Surgery, Johns Hopkins Hospital and University, and Joseph Colt Bloodgood, M.D., Clinical Professor of Surgery, Johns Hopkins Hospital and University. Cloth. Price, \$5. Pp. 709, with 406 illustrations. New York: American Journal of Cancer, 1931.

DONNÉES ACTUELLES SUR L'HORMONE TESTICULAIRE: MODES D'OBTENTION ET DE CARACTÉRISATION. Par L. Cuny, Chef de Travaux, D. Quivy, Assistante, au Laboratoire de Physiologie pathologique de l'École des Hautes-Études (Collège de France). Price, 16 francs. Pp. 76. Paris: Masson et Cie, 1932.

THROMBOSE: IHRE GRUNDLAGEN UND IHRE BEDEUTUNG. Von Professor Dr. A. Dietrich, Direktor des pathologischen Instituts der Universität Tübingen. Price, 8.80 marks; Bound, 10 marks. Pp. 102, with 26 illustrations. Berlin: Julius Springer, 1932.

EPIDEMIC ENCEPHALITIS: ETIOLOGY; EPIDEMIOLOGY; TREATMENT. Second Report by the Matheson Commission: William Darrach, Chairman, Haven Emerson, Frederick P. Gay, William H. Park, Charles R. Stockard, Frederick Tilney, Willis D. Wood, Hubert S. Howe, Secretary, Josephine B. Neal, Executive Secretary, Helen Harrington, Epidemiologist. Price, \$1.50. Pp. 155. New York: Columbia University Press, 1932.

## HISTOGENESIS OF ATROPHIC CIRRHOSIS

V. H. MOON, M.D.

PHILADELPHIA

Atrophic cirrhosis will be used as a term synonymous with alcoholic, Laënnec's, nodular and portal cirrhosis. It includes a somewhat diversified group of pathologic conditions the etiology and mode of development of which have remained an enigma. Many believe that cirrhosis is essentially a chronic inflammatory process. Some authors use the term "chronic interstitial hepatitis" as covering the various forms of cirrhosis. Others discuss cirrhoses in their chapters on chronic inflammatory conditions. Such conditions have an incipience, a period of activity and progression and oftentimes a stage of latency or of healing. If a study on tuberculosis were undertaken, it should include not only the advanced, chronic and perhaps healed lesions; a study of the early, active lesions would be equally instructive. A correct conception of syphilitic disease could not be established by a study of its healed or latent effects in the tissues. By viewing only the charred ruins of a building one might fail to discover the point of origin and the cause of the fire.

Hall and Ophüls<sup>1</sup> found opportunity for studying active cirrhosis by selecting, from 150 necropsies on cirrhotic persons, four cases in which death resulted from other causes and in which the cirrhotic process was in a progressive stage. The subjects in these cases ranged from 28 to 48 years of age. In each of them the liver was larger than normal. Hall and Ophüls concluded that active cirrhosis has the character of a subacute proliferative inflammation with edema, leukocytic infiltration and proliferation of connective tissue, that destruction of liver cells and connective tissue proliferation occur simultaneously as the result of a common cause, and that regeneration of liver cells is a prominent feature.

Active cirrhosis has not received the close study that it merits. It has been my privilege to study material from five cases of atrophic cirrhosis occurring in children under 15 years of age, in which the process was recent and very active, and to study sixteen cases occurring

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From the Department of Pathology, Jefferson Medical College.

Aided by funds from the Martin Research Foundation.

Presented before the Pathological Society of Philadelphia, Dec. 10, 1931.

1. Hall and Ophüls: *Am. J. Path.* 1:477, 1925.



in persons between the ages of 15 and 40, in which the process was less acute. A comparison of features seen in these with those in cases occurring in the later decades of life has been instructive. The histories and postmortem observations in two of the cases have been published.<sup>2</sup> One of these was the case of J. S. He was one of a family of fourteen children, eight of whom were living. There was a history of scarlet fever among these children, but the parents believed that J. S. had not had it. He had never taken alcohol. An older sister had died of atrophic cirrhosis at the age of 13. Three weeks after the death of J. S., a younger brother entered Jefferson Hospital with similar manifestations. An unquestioned clinical diagnosis of cirrhosis was made. His death a few months later occurred outside the hospital. No postmortem examination was permitted. This occurrence of cirrhosis in three children in the same family has some significance.

#### HISTOLOGY OF ACTIVE CIRRHOSIS

The liver of this boy (J. S.) weighed 1,330 Gm.; the boy weighed less than 90 pounds (40.8 Kg.). Histologic examination showed nodules of liver cells surrounded and invaded by recently formed and actively proliferating fibrous tissue. These nodules ranged from 0.2 mm. to 10 mm. in diameter. They consisted of liver cells showing no evidence of compression or of pressure atrophy. The cells were above normal size and showed varying stages of acute degeneration. Some were swollen, and the cytoplasm was finely granular; others contained irregular clumps and masses of hyalin-appearing material that stained more densely than normal cytoplasm. This feature has been emphasized by Mallory<sup>3</sup> as an essential characteristic of this type of cirrhosis. Hall and Ophüls verified this feature in each of their cases. Other cells containing hyaline clumps had lost their nuclei and were in varying stages of disintegration (fig. 2). A study of this feature in many sections of active cirrhosis leads to the conclusion that it is an evidence of far advanced degeneration of the cells. If those showing it are not already necrotic, they are certainly nearing that end.

The great majority of nodules contained some cells which lacked visible nuclei and which had cytoplasm that showed irregular margins. Varying stages of cellular disintegration were evident. These were found about the margins of the nodules, or extending irregularly into them from the periphery (figs. 1 and 3). Proliferation of fibrous tissue and of capillaries from the adjacent stroma appeared where disintegration of liver cells was evident. It was seen adjacent to cells

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2. Moon, V. H.: *Am. J. M. Sc.* **177**:681, 1929.

3. Mallory, F. B.: *Principles of Pathologic Histology*, Philadelphia, W. B. Saunders Company, 1914, p. 505.

showing advanced degeneration. It was not seen among or adjacent to liver cells that were nearly normal. Numerous nodules were present showing all stages of destruction of liver cells and fibrous tissue replacement. Figure 3 shows a nodule with destruction of liver cells and fibrous tissue replacement about the periphery. Figure 4 shows nodules in which only a few liver cells remain, and figure 5 shows an area from which all liver cells have disappeared and in which they have been replaced by newly formed scar tissue containing many bile ducts. It should be emphasized that such areas do not show the connective

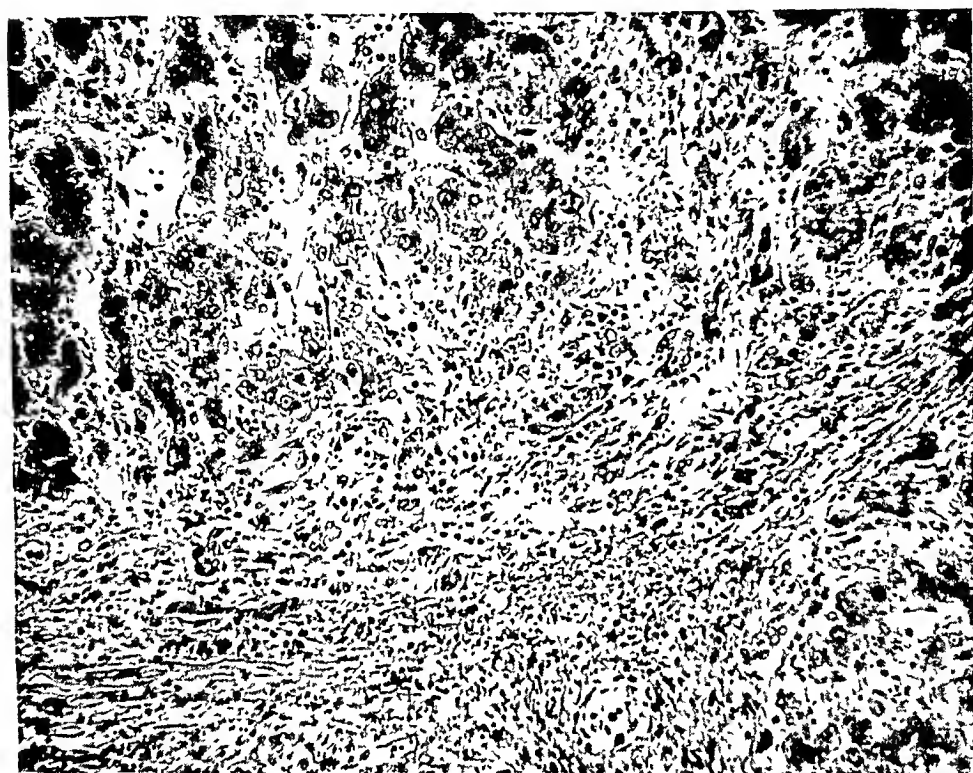


Fig. 1.—Active cirrhosis, the margin of a nodule. Degeneration and disintegration of liver cells and inflammatory infiltration and proliferation of fibrous tissue are shown;  $\times 200$ .

tissue cells and fibrils lying parallel in strands. That feature appears to be produced by compression. As liver cells in uninjured groups proliferate actively they produce an expanding nodule that compresses the soft, newly formed stroma into a parallel position as shown in figure 6. Later these bands may contract causing atrophy of the enclosed liver cells, as seen in later stages of cirrhosis. Such a process explains the development of globular nodules with bands of connective tissue extending concentrically about them, which is the characteristic architecture of Laënnec's cirrhosis.

There has been discussion as to the sequence of events leading to the perinodular fibrosis. Ghon<sup>4</sup> and other European pathologists maintained that the fibrosis precedes the degeneration and destruction of liver cells. Some believe that the destruction of liver cells occurs first, and that the fibrosis follows. Still others, avoiding controversy in the absence of definite evidence, suggest that the two processes occur simultaneously. The latter statement is true as pertaining to the liver in general or to representative sections of it. Destruction of liver cells is seen; proliferation of connective tissue is seen; they occur simul-

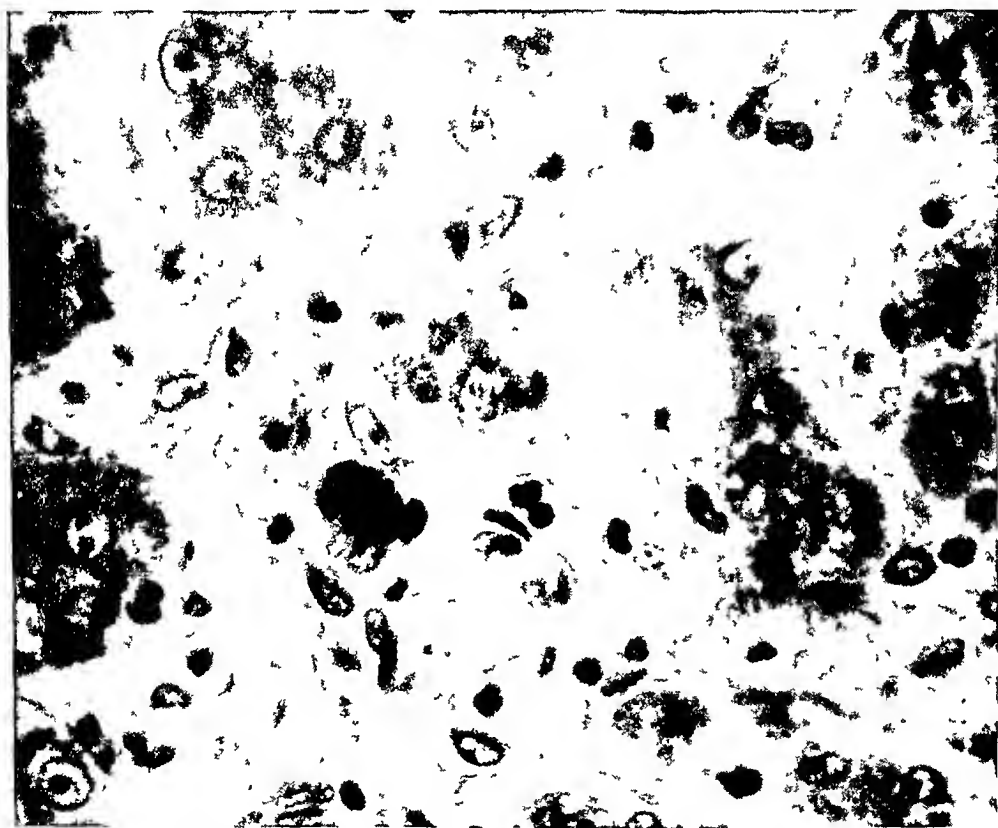


Fig. 2—Active cirrhosis, an area near the margin of a nodule. Heavy, lumpy masses, hyaline in character, are seen in liver cells. Edema, debris of cells, polymorphonuclear and mononuclear cells are present. A few fibers of newly formed connective tissue are shown;  $\times 800$ .

taneously in that sense. But observations of such areas show that the cells are undergoing destruction and cytolysis just a little beyond the margin of connective tissue proliferation (fig. 1). This observation may be verified an infinite number of times in sections from active cirrhosis. I have not seen instances in which the connective tissue

<sup>4</sup> Ghon, A., in Aschoff, I. *Pathologic Anatomy*, ed 7, Jena, Gustav Fischer. 1928, vol 2, p 886

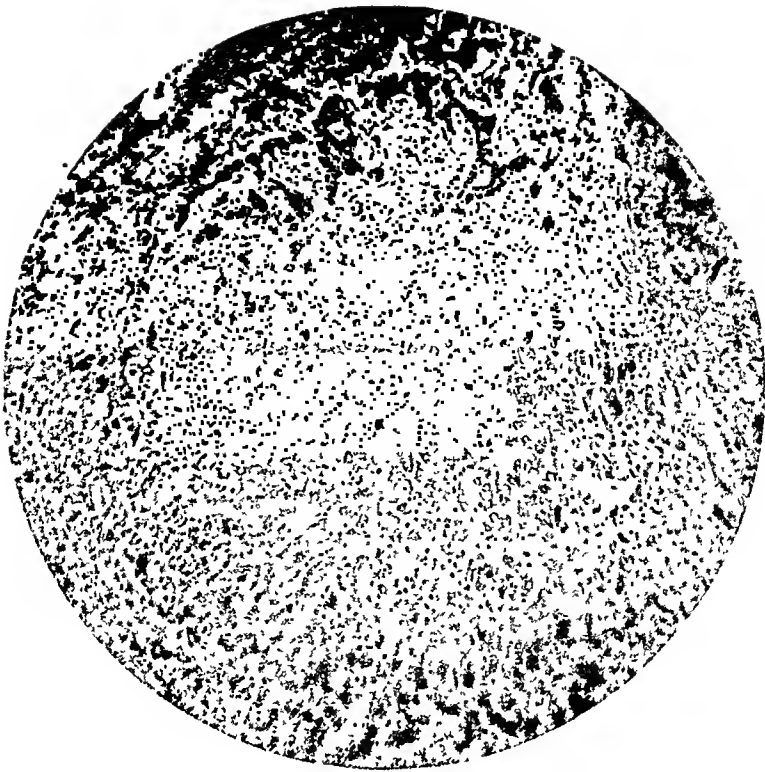


Fig. 3.—Active cirrhosis, small nodule, the margins of which are undergoing destruction and replacement. Numerous bile ducts are seen; some are atypical and resemble syncytial masses;  $\times 100$ .

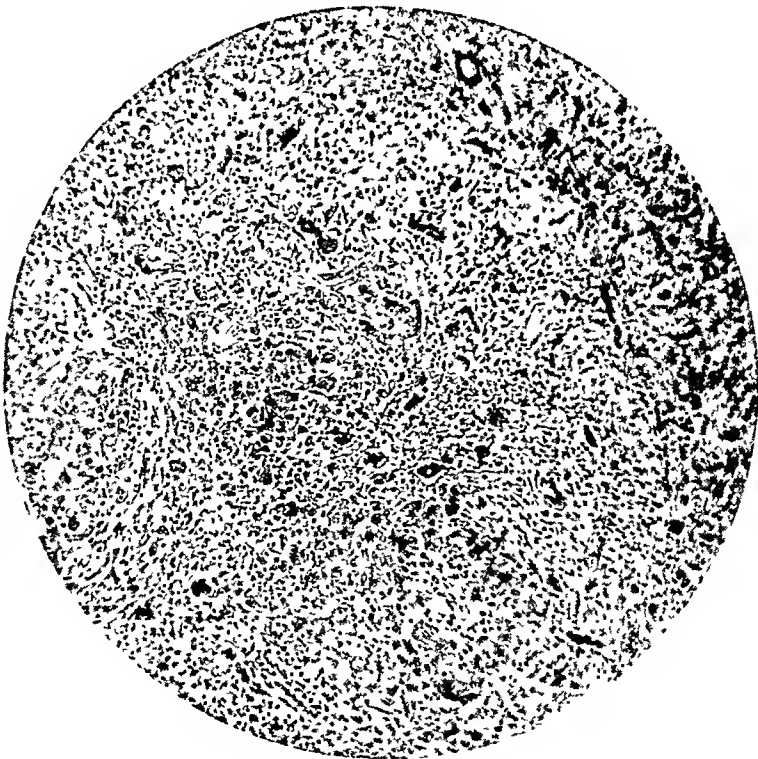


Fig. 4.—A nodule in which destruction and replacement are more advanced than in figure 3;  $\times 100$ .



Fig. 5.—A nodule from which all liver cells have disappeared. This is soft granulation tissue containing many bile ducts. Such fibrous tissue is easily compressed into a band of parallel fibers by the expansive growth of an adjacent hyperplastic nodule;  $\times 100$ .

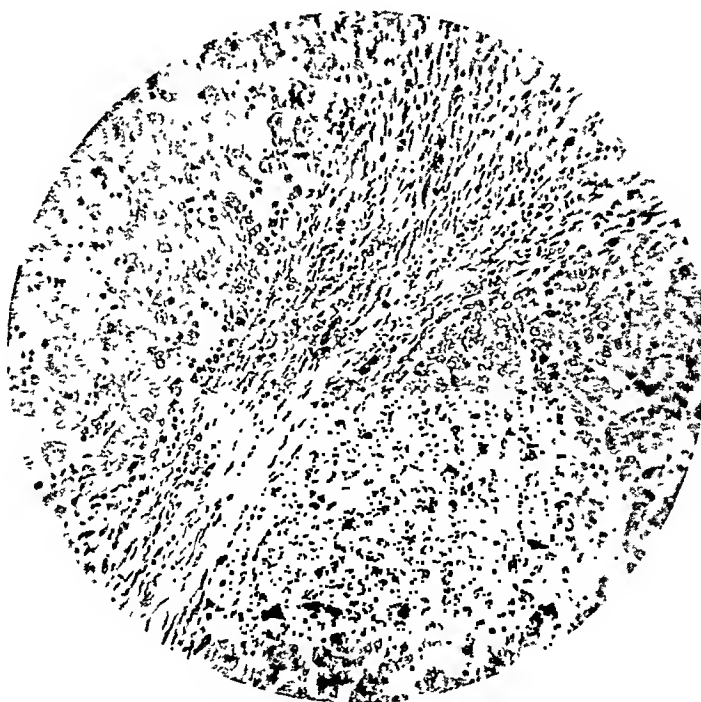


Fig. 6.—Hyperplasia of uninjured groups of cells produces expanding nodules, which compress the soft fibrous tissue into bands. These nodules are presently destroyed by action of the injurious agent. Degeneration is beginning in the nodule at the left. Later it would resemble that shown in figure 3;  $\times 100$ .

proliferation was not perceptibly behind the degeneration and destruction of liver cells. The process is similar to that in other instances of progressive inflammation. Destruction of parenchymatous tissue occurs first, and replacement with fibrous tissue follows.

Other observers have made a similar interpretation. MacCallum<sup>5</sup> gave a concise description of the process:

The liver-cells are killed in patches—whole lobules and groups of lobules at a time, or only parts of lobules. There remain irregular masses of liver tissue partly disconnected from their bile ducts. . . . The masses of liver-cells quickly increase in size by multiplication of their cells, new capillaries are formed in every direction, and this labyrinth of cells expands, pressing the stroma away on all sides. For a time the liver-cells are normal, but then comes another injury, and many of the hyperplastic nodules are partly destroyed. The whole process is repeated, and not only once, but many times. It is clear that this must lead to an extraordinary distortion of the liver's structure. There are no longer lobules, but only nodules produced by the hyperplasia of smaller groups of cells which were left intact.

This is directly contrary to the supposition that the cirrhotic process is primarily one of fibrous tissue proliferation, and that reduction of liver parenchyma results from the contraction of the fibrous tissue. Studies of sections from active cirrhosis reveal no evidence to support such a supposition. They tend to support MacCallum's conception.

The proliferation of bile ducts is a prominent feature in active cirrhosis. Many bile ducts appear normal in structure. Others (fig. 3) are atypical and resemble syncytial masses irregular in form and having no lumen nor even a tubular arrangement. Not all authors are agreed that actual proliferation of bile ducts occurs. Some believe that the apparent increase is merely relative; that, with the disappearance of liver lobules, the bile ducts located at their periphery are brought closely together. The numerous bile ducts seen in figures 4 and 5 do not support such a supposition. These ducts lie in all planes, and their long axes extend in every direction. Many cross each other at various angles. Such positions would not be produced by compression of liver substance resulting from destruction of lobules. An actual count was made of the bile ducts seen in sections of normal and cirrhotic livers. A section of normal liver 1 cm. square and 5 microns thick, contained from 340 to 470 visible bile ducts. A similar area of cirrhotic liver contained from 4,790 to 6,640 bile ducts. The cirrhotic liver contained about fourteen times as many bile ducts per gram as were found in normal liver. The cirrhotic liver was larger than normal for a subject of similar body weight. Proliferation of bile ducts seems the only adequate explanation for such an actual increase.

The cellular infiltration in areas of active destruction of liver cells and new tissue growth consisted almost entirely of polymorphonuclear

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5. MacCallum, W. G.: *Text-Book of Pathology*, ed. 4, Philadelphia, W. B. Saunders Company, 1928, p. 304.

cells. A few monocytes and lymphocytes were present. No abscesses or areas of dense inflammatory infiltration were seen in any part of the liver. The infiltration within the bands of fibrous tissue showed a higher percentage of lymphocytes than was seen in areas of active inflammation.

Studies on each of three other cases of cirrhosis occurring in children showed no essential differences from the features seen in the case of J. S. There were minor variations in the acuteness and extent of injury, but the same salient features were exemplified in each of the four actively cirrhotic livers.

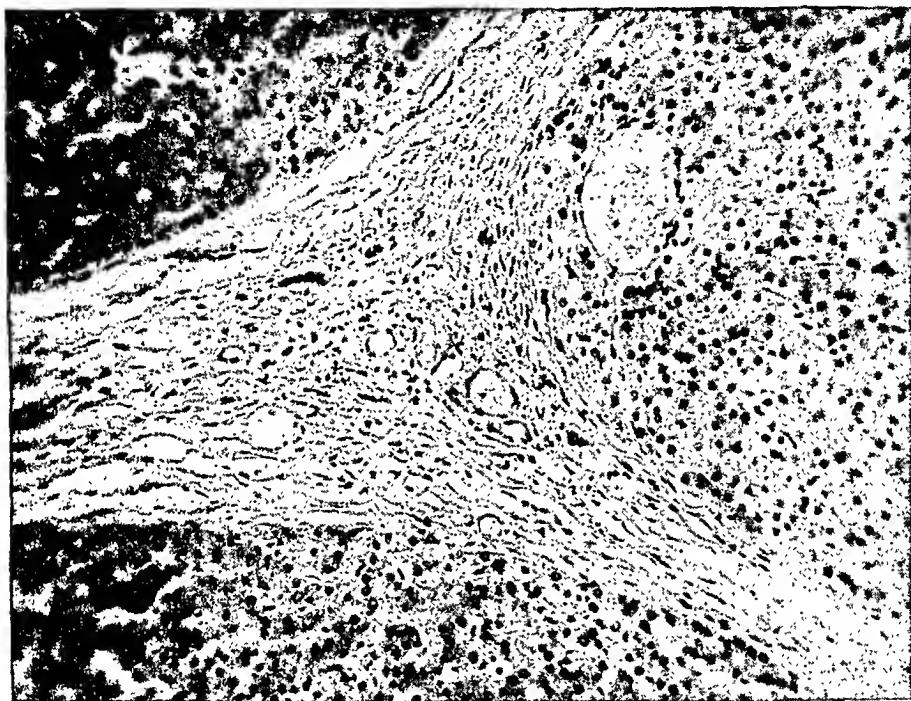


Fig. 7.—Healed cirrhosis from a child, 9 years old. There is no degeneration of liver cells or inflammatory reaction. The bands consist of mature fibrous tissue, none of which is in process of formation. No organisms were found in this liver;  $\times 200$ .

The fifth case was that of a child 5 years of age in which enlargement of the liver and spleen with progressive anemia was followed by splenectomy with recovery. Four years later the child died of a malignant tumor associated with giant follicular lymphoid hyperplasia. Postmortem examination showed marked atrophic cirrhosis in addition to the malignant tumor.<sup>6</sup> The histologic features presented a sharp contrast to the active process seen in the four cases described. Large

6. Dr. B. S. Kline, Mount Sinai Hospital, Cleveland, provided me with the clinical and postmortem data in this case and gave me an opportunity to compare sections from it and from an additional case with those in the other cases studied.

nodules of liver cells were enclosed by dense bands of connective tissue, which was mature (fig. 7). The fibers were typically elongated and lay parallel in compact strands which contained vessels and bile ducts that appeared normal. The liver cells showed no evidence of degeneration and destruction. There were no hyaline masses in the cytoplasm, no leukocytic infiltration and no recent proliferation of connective tissue. The lobular architecture of the liver was distorted somewhat, but the cells lay in strands as normally. The lobular veins were eccentrically located.

This was a case of healed cirrhosis occurring in childhood following splenectomy. Removal of the spleen may have removed an important causative factor before irreparable damage was done to the liver. This supposition is in keeping with surgical experience. Splenectomy in early stages of cirrhosis may interrupt the process, and recovery may follow.

#### HISTOLOGY OF LESS ACTIVE CIRRHOSIS

Atrophic cirrhosis occurring in children is more acute and active than that seen in adults. Cirrhosis occurring in early adult life usually shows histologic evidence of greater activity than that seen in later decades of life. The clinical manifestations show a corresponding variation. The course is more rapid, and the physiologic disturbances are more acute in the earlier than in the later periods. Each of the four cases of active cirrhosis seen in children ended fatally within a year from the onset of the disease. The case of J. S. ran its complete course in ten weeks. The cases occurring before the age of 40 usually run their course in from one to three years. Cases developing after the age of 40 are usually more chronic and may last for from two to ten years. These generalizations, drawn from a review of clinical histories, admit exceptions. The insidious beginning of cirrhosis, especially in adults, makes impossible any precise statements concerning the duration of the disease.

The histology of cirrhotic livers in young adults shows the same characteristics of a productive inflammatory process as those in children. There are progressive degeneration and necrosis of liver cells. The degenerated cells contain irregular masses of hyalin-appearing substance. There is evidence of progressive destruction of groups of liver cells and replacement with fibrous tissue (fig. 8). Fibroblasts and recently formed fibrous tissue are seen in such areas. The bands that characteristically enclose islands of liver cells are more dense and apparently consist of more mature fibrous tissue than that seen in active childhood cirrhosis.

The character of the cellular infiltration varies widely. Polymorphonuclear cells are numerous about the areas where liver cells show



degeneration and necrosis and where proliferation of fibrous tissue is active. Lymphocytic and plasma cell infiltration is more prominent in adult than in juvenile cirrhosis, and the process shows other evidences of greater chronicity. The cirrhotic process is active, but the rate of progress appears to be slower.

#### CIRRHOSIS WITH FATTY CHANGES

Frequently fatty changes of varying degree are associated with cirrhosis. These have been interpreted variously. Many European pathologists classify fatty cirrhosis separately. Most English and



Fig. 8.—Moderately active cirrhosis in a man, aged 54. The bands consist of mature fibrous tissue. Active destruction of liver cells, inflammation and connective tissue proliferation are seen, but not so extensively as in very active cirrhosis. No organisms were found in this liver;  $\times 200$ .

American writers discuss it as a variation of atrophic cirrhosis. The cases of juvenile cirrhosis studied showed no associated fatty changes. Such changes were found frequently in adults. The degree of fatty change varied exceedingly. In most cases, some cells were found containing visible fat globules. In perhaps 30 per cent of the cases of adult cirrhosis there was a rather marked amount of fatty change, and in a few the fatty changes were so marked as to dominate the histologic picture. In such livers, the characteristic features of active cirrhosis were not different from those already described (fig. 9). There was

evidence of progressive degeneration and destruction of liver cells. Many contained irregular masses of hyaline matter. Cellular infiltration and proliferation of fibrous tissue were prominent. The injury or combination of injuries that causes cirrhosis may be expected to affect fatty livers as readily as those containing no fat. Fat may accumulate more readily in a damaged liver than in a normal one. The progressive degenerative changes occurring in the cells as a part of active cirrhosis may include varying degrees of fatty degeneration and infiltration. This would be more likely to occur in the more slowly progressing cases. Fatty changes are seen frequently in chronic cirrhotoses occurring

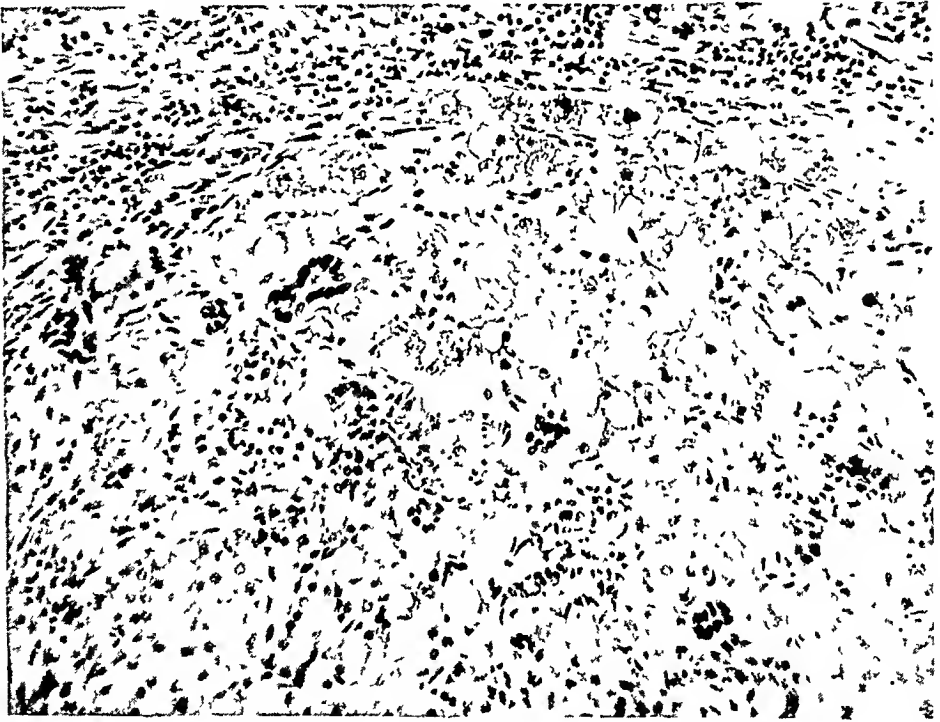


Fig 9—Very active cirrhosis in a fatty liver; marked degeneration and destruction of liver cells; active inflammation and proliferation. Hemolytic streptococci were cultivated from this liver;  $\times 400$ .

in adults, especially in those addicted to alcohol. They are not seen in the rapidly progressing cirrhosis of childhood.

There seems to be no justification for a separate classification of cirrhosis on the basis of fatty changes.

#### LATENT OR HEALED CIRRHOSIS

Frequently postmortem examination reveals a typically cirrhotic liver in a case that gave no clinical evidence of that condition. McCartney <sup>7</sup>

7. McCartney, J. S., Jr. Abstr, Am J. Path 7:572, 1931.

reported a clinical and pathologic study of 297 cases of cirrhosis. He stated that about two thirds of these were clinical and one third latent. In a comparison of sections, particularly from patients of advanced age, one sees marked variation in the activity of the cirrhosis. In some cases there is evidence as described, of progressive cirrhosis. In others such evidence is slight or is entirely absent. No progressive degeneration of liver cells is seen. There are no hyaline masses in the cytoplasm. There is no polymorphonuclear infiltration. Connective tissue proliferation is slight. The dense bands of fibrous tissue that surround nodules of liver cells may contain a few or many lympho-

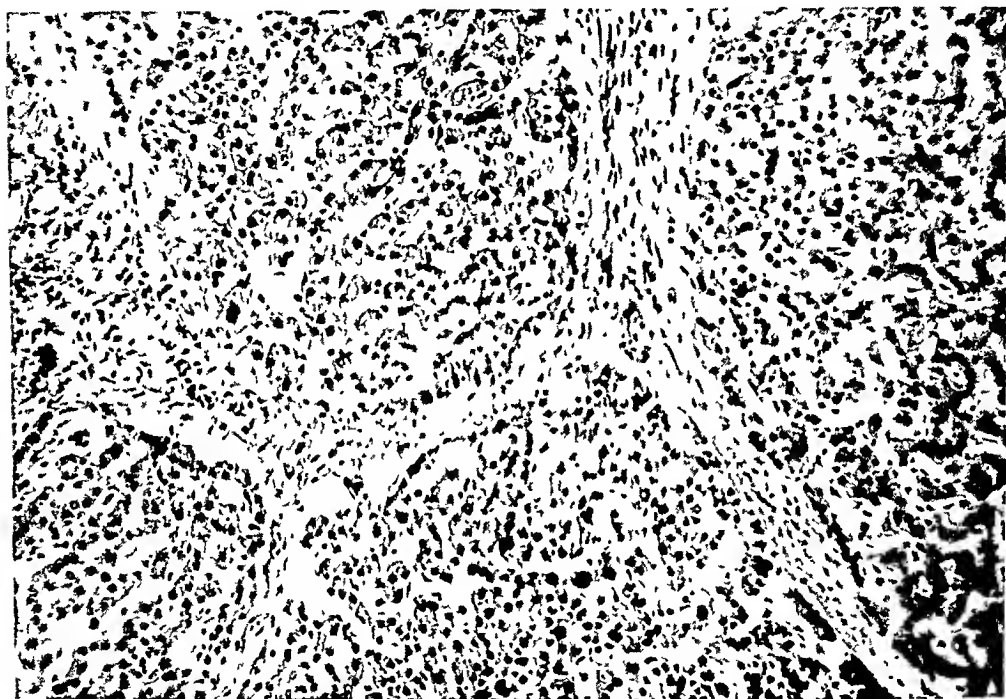


Fig. 10.—Healed cirrhosis in a man, aged 67, who showed no clinical evidence of cirrhosis. Death occurred from pneumonia. There is no degeneration of liver cells, inflammatory reaction or recent growth of fibrous tissue. No organisms were found in this liver;  $\times 150$ .

cytes, but it is mature, adult fibrous tissue. Many of the liver cells may contain pigment, and they may be irregular in size and shape as if showing atrophy from pressure. In some cases, the liver cells may even be arranged in strands with well defined sinusoids between them (figs. 7 and 10).

I reviewed the stained sections from forty-four recent postmortem examinations on adults in which atrophic cirrhosis was found. Without reference to the clinical histories, these were classified as to the activity of the process. In every instance in which histologic evidence of activity was slight or was absent, reference to the clinical history

showed that there had been no ascites, no tendency to bleed, no splenic enlargement nor other signs of cirrhosis, and a clinical diagnosis of cirrhosis had not been made. There were instances, however, in cases not recognized clinically as cirrhosis, in which there was histologic evidence of activity. The activity in these cases was less marked than in the cases recognized clinically as cirrhosis.

The histologic evidence indicates that cirrhosis is the result of a progressive inflammatory process having a proliferative rather than an exudative character. This process is seen in its most active form in cases occurring in children. The inflammatory process presents more evidence of chronicity in young adults, and still more such evidence in the later decades of life. There are histologic evidences of healing or of latency of this chronic inflammatory process. Such evidences are seen most frequently in subjects over 50 years of age.

Mallory laid stress on the hyaline masses seen in the cytoplasm of the liver cells. He regarded them as characteristic and diagnostic of this type of cirrhosis, for which he uses the term alcoholic, although he does not claim that alcohol is anything more than a contributory cause. I have found these hyaline masses in each case of active cirrhosis regardless of the age of the subject. They are numerous in the very active cirrheses of childhood. They are less numerous in sections from less active cirrhosis, and I have not found them in latent or healed cirrhosis. I regard them as evidence of the activity of the process, and their number as one of the indexes of activity, rather than as a diagnostic feature for atrophic cirrhosis as a type.

A pathologic condition of the spleen was a regular feature in all the cases of active cirrhosis that I have seen. The character and significance of this will be discussed in a later communication.

The active inflammatory character of progressive cirrhosis suggests infection as a probable cause. It is difficult to produce such a process by the use of intoxicating agents alone. Opie<sup>8</sup> produced cirrhosis experimentally by a combination of intoxicating agents and infection. Forms of cirrhosis have been produced by infectious agents alone. Attention was called recently<sup>2</sup> to infection as a cause of juvenile cirrhosis. In that article, observations and experiments of others were cited supporting the supposition that infection may be an important etiologic factor. The case of J. S., referred to in the present article, was one of those in which streptococci were cultivated from the liver as reported.

Since the publication of that paper I and my associates, F. W. Konzelmann and H. L. Stewart, have cultivated hemolytic streptococci from the livers in five cases of active cirrhosis. Details of these cases

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8. Opie, E. L.: *J. Exper. Med.* **12**:367, 1910.

and of experiments based on them will be reported later. Frequently bacteria may be found in large numbers in sections from active cirrhosis appropriately stained. Paraffin sections of liver preserved by Zenker's method are suitable. Sections should be 5 microns or less in thickness and should be stained either by Gram's technic or by the Gram-Weigert modification. The areas of degenerated liver cells in the nodules of active cirrhosis usually contain a few cocci. Frequently these are present in large numbers. Their grouping is irregular. They occur in pairs, in short chains of six or eight, and in irregular clumps. They may be seen in disintegrating or in degenerating liver cells and in the intercellular spaces. A few are seen within leukocytes. Phagocytosis is not marked in such tissues. They are rarely seen in the areas of connective tissue proliferation. They are found in largest numbers in the very active cirrhosis of childhood and in smaller numbers in early adult cirrhosis. I have found them only occasionally, and then in small numbers, in moderately active cirrhosis in adults. I have not found any bacterial forms in the latent or healed stages of cirrhosis.

MacMahon and Mallory<sup>9</sup> reviewed the literature on streptococcus hepatitis, and reported five cases in four of which they demonstrated streptococci in the livers. The fifth they regarded as a healed streptococcal lesion. Two of the cases had the features of acute degeneration and necrosis of liver cells and an inflammatory and reparative response resembling that which I have described as occurring in active cirrhosis. These might possibly be cases of cirrhosis in an incipient stage. A third case was one in which a section of liver, excised during cholecystotomy, was normal histologically. Subsequently chills and fever developed, later jaundice appeared and finally ascites, which was tapped on two occasions. The liver at this time was definitely enlarged. The patient's condition progressed, and eight and one-half months after the operation he died with extensive cirrhosis of the liver. The authors' histologic description corresponds to that which I have given for active cirrhosis. Numerous streptococci were seen in sections of the liver. They regarded it as a chronic lesion due to streptococci of moderate virulence causing widespread gradual necrosis with fibrous tissue proliferation. They suggested a possible relationship between streptococcus infection and both acute yellow atrophy and cirrhosis, but refrained from drawing unwarranted conclusions.

Mallory<sup>10</sup> and Adami<sup>11</sup> reported the finding of bacilli in cirrhotic livers. Thus far I have found only coccal organisms. Hall and Ophüls<sup>1</sup> examined sections, stained by Giemsa's method, from four cases of

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9. MacMahon, H. E., and Mallory, F. B.: *Am. J. Path.* **7**:299, 1931.

10. Mallory, F. B.: *Bull. Johns Hopkins Hosp.* **22**:69, 1911.

11. Adami, J. G.: *Lancet* **2**:396, 1898.

active cirrhosis, but saw no bacterial forms. I have had little success in demonstrating bacteria in tissues by Giemsa's method. Perhaps my technic was imperfect, or perhaps that method is less effective than Gram's method.

Cocci were found in large numbers in sections from four cases of juvenile cirrhosis (fig. 11). They were found in smaller numbers in nine cases of cirrhosis in adults. No cocci were found in the liver of the child whose cirrhotic process was interrupted by splenectomy, nor in any case of latent or healed cirrhosis in adults.

Studies of sections of active cirrhosis have made inescapable the conclusion that it is a progressive inflammatory process. Such processes are most frequently the result of infection. The infectious character of

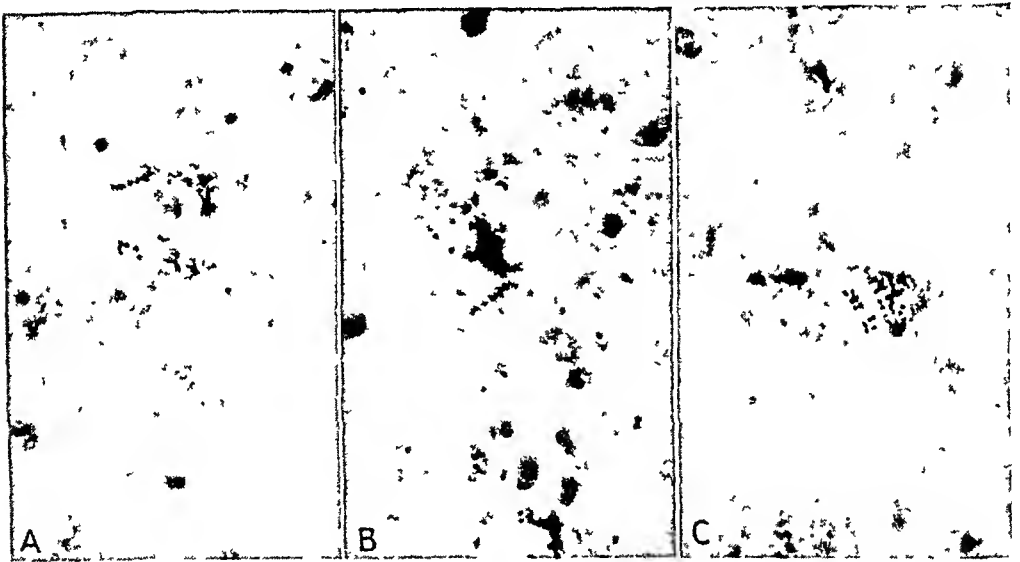


Fig. 11.—Sections from three cases of very active cirrhosis seen in children. Hemolytic streptococci were cultivated from the livers A and B. No cultures were made from C. Cocci are most numerous in the debris of disintegrated liver cells. A few are seen in other liver cells and within phagocytes. Zenker fixation; paraffin sections; Gram stain;  $\times 1,500$ .

cirrhosis has been suggested by many previous observations. In view of these, the frequent demonstration of organisms in cirrhotic livers is of interest. The finding of cocci in such livers should be as significant as the finding of spirochetes in cases in which a syphilitic origin was suspected.

#### CONCLUSIONS

Atrophic cirrhosis is the result of a progressive inflammatory process, proliferative rather than exudative.

This process shows variation in activity and may be seen in latent or healed stages. The most active process is seen in juvenile cases. The latent stages are seen most often in adult life.

The acuteness or latency of the process frequently parallels the clinical manifestations.

There is evidence, in active cirrhosis, that degeneration and destruction of liver cells precedes the replacement by connective tissue.

No morphologic reason is found for placing cirrhosis with fatty changes in a separate class.

Cocci may be demonstrated frequently in sections of active cirrhosis. This observation lends support to the supposition that infection is an important etiologic factor.

# EFFECT OF DIACETONE ALCOHOL ON THE LIVER OF THE RAT

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In a study in rabbits of experimentally produced convulsions and their prevention<sup>1</sup> it was noted that of the four derivatives of acetone tested, diacetone alcohol had the most marked anticonvulsive effect. This is of particular interest in relation to the effect of ketogenesis on epileptic attacks. Although the explanation of the action of the ketogenic diet is not yet clear, it has been shown that certain of the acetone bodies in themselves have a definite anticonvulsive effect. Before attempting to use diacetone alcohol therapeutically it was necessary to determine its toxic effect. Walton, Kehr and Loevenhart<sup>2</sup> had shown that the maximal tolerated intravenous dose for rats was 3 cc. per kilogram of body weight. I was able to show that, when given by stomach tube, this was also the maximal tolerated dose for rabbits. A much smaller amount (0.5 cc. per kilogram of body weight) was found to be definitely anticonvulsive, with little, if any demonstrable toxic effect.

Since the liver is readily injured by toxic agents, it was determined to observe what changes might occur after a single, moderately large dose of the drug.

## METHOD

Twenty adult white rats were placed in separate cages in pairs and made to fast for forty-eight hours. They were then allowed to feed for two hours, and were again made to fast for twenty-two hours. Then 2 cc. per kilogram of body weight of redistilled diacetone alcohol was administered by stomach tube. Before giving the drug, and at intervals afterward, determinations of the concentration of hemoglobin in the blood (Dare) and erythrocyte counts were made. Pairs of rats were then killed at intervals of from one hour to sixty days. Sections of the liver were fixed for twenty-four hours in Zenker's solution plus a diluted solution of formaldehyde and stained with hematoxylin and eosin and with eosin azure II. Four animals were killed as controls.

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From the Section on Pediatrics, the Mayo Clinic.

Work done in the Division of Experimental Surgery and Pathology, the Mayo Foundation.

1. Keith, H. M.: Proc. Staff Meet., Mayo Clin. **5**:204, 1930; **6**:410, 1931; Am. J. Dis. Child. **41**:532, 1931; J. Pharmacol. & Exper. Therap., to be published.

2. J. Pharmacol. & Exper. Therap. **33**:175, 1928.



## OBSERVATIONS

As evidenced in the accompanying table, there was destruction of erythrocytes, with reduction of the hemoglobin content. This was present for a period of four or five days only; the values for both returned approximately to normal on the sixth day.

Microscopic examination of the liver disclosed no changes one hour after the drug had been given, but at six hours some changes appeared. There were marked activity and increase in the number of lymphocytes in the portal spaces and of the histiocytes of Kuffer cells along the vascular sinusoids; also some slight vacuolization of the hepatic cells characterized the parenchyma in the portal zones. At twelve hours,

*Estimation of Hemoglobin and Erythrocytes of Rats Following One Large Dose of Diacetone Alcohol by Stomach Tube*

Rat	Before Giving Drug		After Giving Drug											
			2 Hr.		4 Hr.		24 Hr.		48 Hr.		4 Days		6 Days	
	Hemoglobin *	Erythrocytes, Millions	Hemoglobin *	Erythrocytes, Millions	Hemoglobin *	Erythrocytes, Millions	Hemoglobin *	Erythrocytes, Millions	Hemoglobin *	Erythrocytes, Millions	Hemoglobin *	Erythrocytes, Millions	Hemoglobin *	Erythrocytes, Millions
1	85	5.37	..	..	..	..	..	..	..	..	..	..	..	..
2	90	5.34	..	..	..	..	..	..	..	..	..	..	..	..
3	98	5.65	70	5.30	..	..	..	..	75	4.88	..	..	..	..
4	95	5.55	90	5.38	..	..	..	..	70	4.96	..	..	..	..
5	88	5.30	..	..	..	..	..	..	76	4.84	..	..	..	..
6	90	5.40	..	..	..	..	..	..	78	4.88	..	..	..	..
7	93	5.60	..	..	..	..	..	78	4.75	74	4.55	..	..	..
8	95	5.85	..	..	..	..	75	4.57	70	4.40	..	..	85	5.22
9	90	5.35	..	..	..	..	70	4.49	66	4.31	73	4.60	80	5.18
10	85	5.32	..	..	..	..	70	4.45	65	4.24	70	4.72	80	5.10
11	90	5.33	..	..	90	5.29	..	..	..	..	..	..	..	..
12	88	5.26	..	..	90	5.30	..	..	..	..	..	..	..	..

\* Per cent (Dare).

these changes were accentuated, and lymphocytic infiltration in the portal spaces had increased. Vacuolization of the cytoplasm of the hepatic cells, first evident at six hours, now involved the entire zone around the portal spaces, causing enlargement of the cells in some cases sufficient to obliterate the sinusoids. Eighteen hours after administration of the drug, vacuolization and granulation had involved from two thirds to three fourths of the lobule, but the number of lymphocytes and littoral histiocytes was even less than at the twelve hour stage.

Maximal injury was present at twenty-four hours. There was extensive cloudy swelling of the hepatic cells throughout the entire lobule, with vacuolization and granulation of the cytoplasm. This was most marked about the nuclei, although the latter were entirely uninjured. Swelling of the parenchymal cells was such as to obliterate the sinusoids completely. The histiocytes were sparse, and only a few lymphocytes could be found.

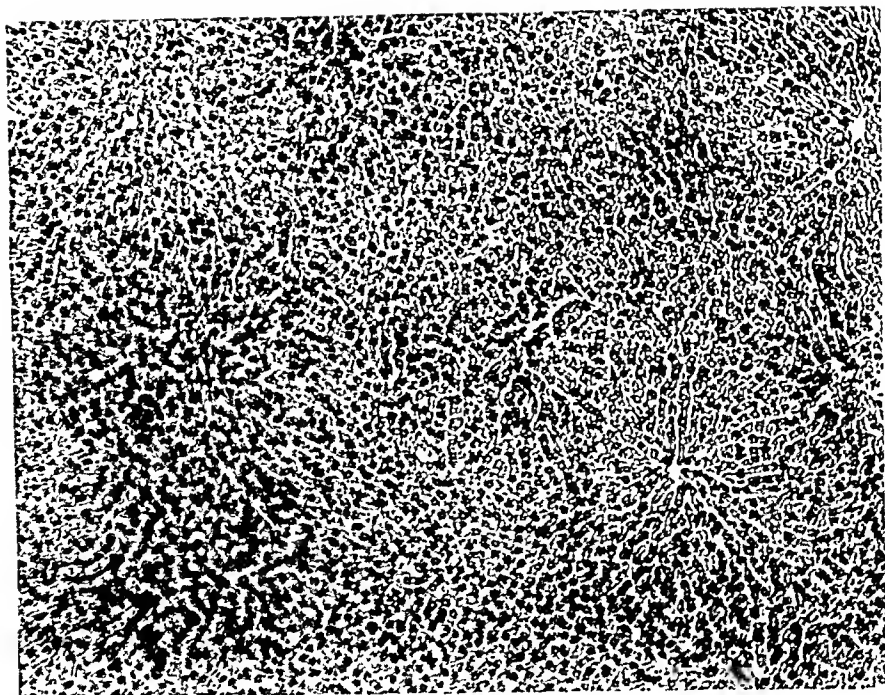


Fig. 1.—Normal liver of rat.

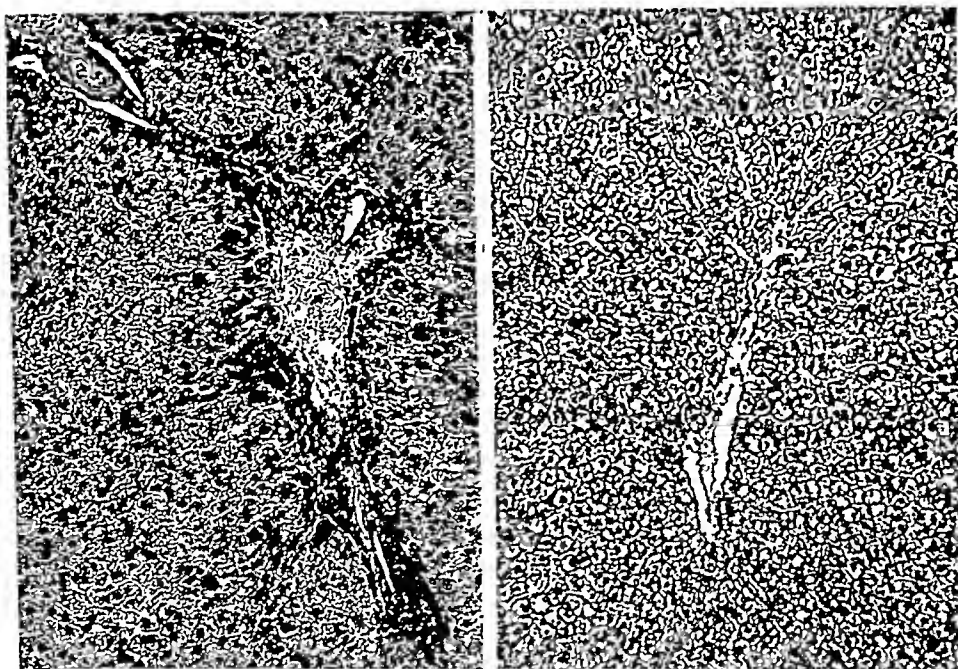


Fig. 2.—On left is shown periportal infiltration twelve hours after administration of diacetone alcohol (2 cc. per kilogram of body weight) given by stomach tube; on right, vacuolization and granulation of hepatic cells in liver of rat twenty-four hours after administration of diacetone alcohol (2 cc. per kilogram of body weight) given by stomach tube.

At forty-eight hours, beginning recovery was evident, particularly in the central portion of the lobule, from which a definite gradient of recovery toward the periphery was seen. Cells in the portal areas, however, still gave evidence of marked injury. The hepatic cells were decreased in size, and the vascular sinusoids reappeared, now containing a few erythrocytes. There was some increase in the number of histiocytes also, but this was not marked.

Recovery was well advanced at ninety-six hours, especially in the central and medial portions of the lobules. Here the cytoplasm was homogeneous, although there was some granulation still present at the

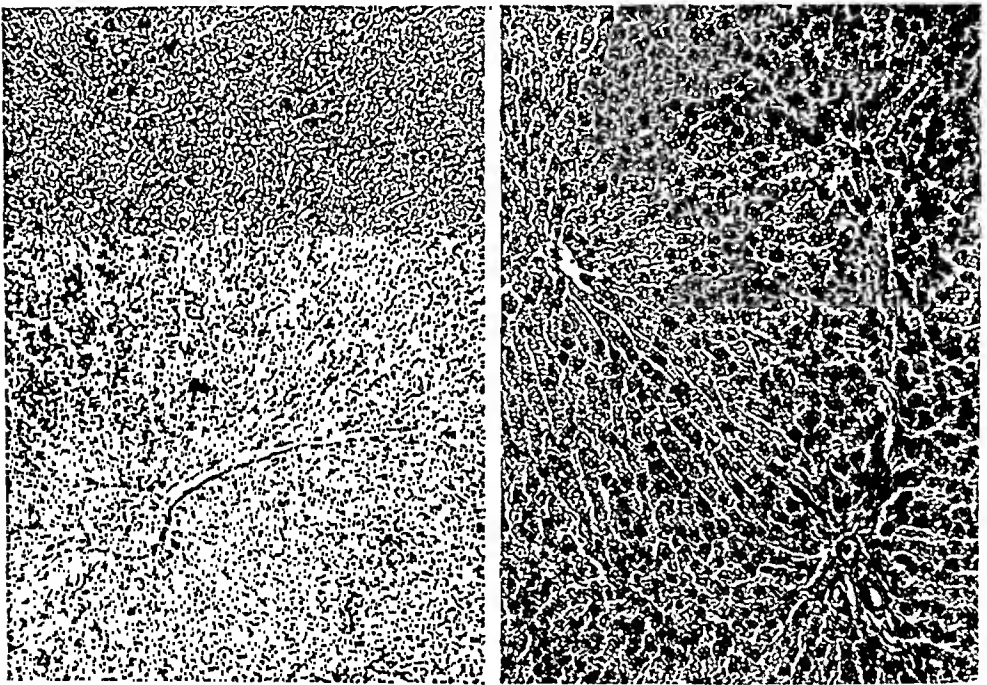


Fig. 3.—On left is shown beginning recovery of liver of rat forty-eight hours after administration of diacetone alcohol; on right, almost complete recovery of liver of rat ninety-six hours after administration of diacetone alcohol.

periphery. The sinusoids were normal throughout the lobule, but the number of histiocytes was much increased.

At seven and fourteen days, the hepatic cells were practically normal, although some granulation still persisted in the portal areas. At fourteen days, large numbers of histiocytes had appeared, and at twenty-one days these had increased to a remarkable extent.

Scattered throughout the lobules were large numbers of "nests" of these phagocytic cells, containing also nucleated erythrocytes. This presented the picture of so-called "myeloid metaplasia." The hepatic cells were, however, normal both in size and in organization. At thirty-five

days, there was no evidence of injury, and the histiocytes were again normal in number and distribution.

Two animals were killed at each interval throughout the experiment, and the changes in the two series were essentially identical. At seven days, the hepatic cells of the animal of one series had apparently recovered to a greater extent than those of the corresponding animal of the other series, and at twenty-one days there was a difference in the extent of the histiocytic reaction between the two. These, however, were only differences in degree, for the type of change was constant and readily recognizable.

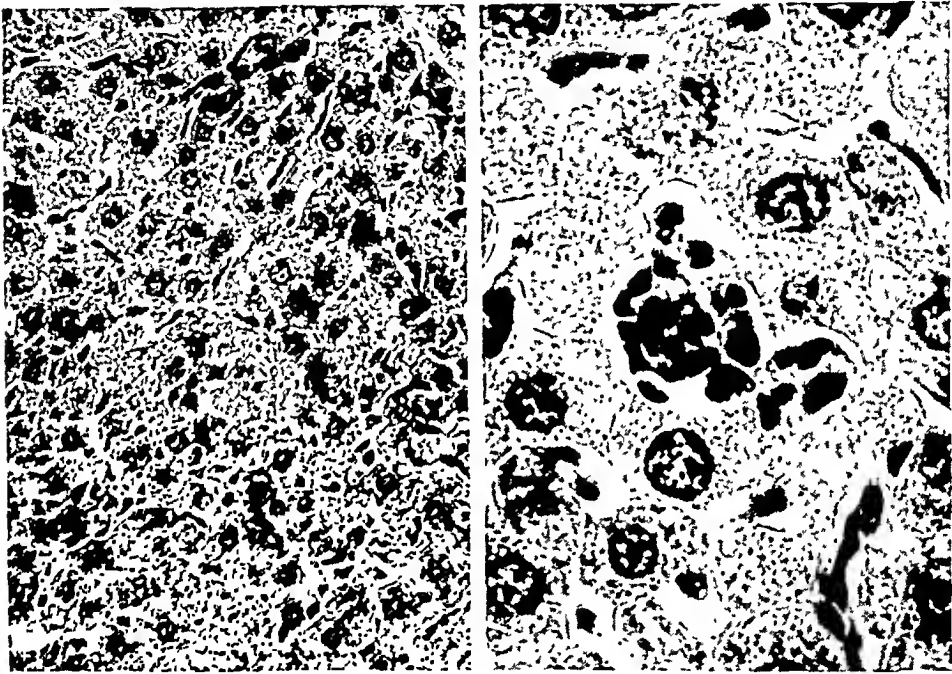


Fig. 4.—On the left are shown histiocytes in liver of rat twenty-one days after administration of diacetone alcohol; on the right, a higher magnification.

#### COMMENT

From the observations related, it is evident that diacetone alcohol in large quantities has two definite effects: first, destruction of erythrocytes and reduction in the amount of hemoglobin; second, injury to hepatic cells, with rapid recovery. These effects were followed by marked histiocytic proliferation. Although the amount of the drug given was fairly large, and the animals became drowsy for a few hours, they did not appear to be greatly affected otherwise. The destruction of erythrocytes was definite although not extensive, and both determinations of hemoglobin and erythrocyte counts were close to their previous values on the sixth day.

Injury to the hepatic cells commenced within six hours following injection of the drug, and reached its maximal degree at about twenty-

four hours, involving all the cells in the lobule. Recovery began within forty-eight hours, was well advanced in four days, and was practically complete in seven days. The injury to the parenchymal cell, therefore, was not severe.

The reaction of the local histiocytes in the liver is of considerable interest. Normally, these Kupffer cells, or phagocytic cells, are not abundant in the rat, but are scattered at intervals along the sinusoids, acting as scavengers and hemophages and contributing their share to the normal physiology of the organ. In the absence of the spleen, or after other destruction of the reticulo-endothelial structures, notable compensation occurs in the liver, so that these reticular cells rapidly increase in size and number, and often give rise to small foci with apparent hematopoietic capacity. The histiocytic reaction in the liver following administration of diacetone alcohol is not unlike that occurring in certain other instances of marked hyperplasia of this system. Early responses to the drug include destruction and disappearance of these cells as well as of the parenchymal cells. Subsequently, however, and coincident with repair of the lobule, new histiocytes appear from an apparently extraneous source. There is no evidence to warrant the conclusion that these vast numbers of histiocytes arise locally. Their rapid increase did not cease at the time normal parenchyma was restored, but continued well after three or four weeks had elapsed. In many instances, these proliferating histiocytes were arranged in nests of cells scattered along the sinusoids, and in some of these nucleated erythrocytes were identified. This hematopoietic activity of the reticular cells, appearing at twenty-one days after injection, and long after the erythrocyte and leukocyte counts had returned to normal, indicates, it would seem, a reaction to a persistent stimulus within the blood stream. The hemolytic action of the drug no doubt had thrown an excessive load on the normally active hematopoietic centers, such as the bone marrow; the newly formed histiocytes in the liver, reacting to the identical stimulus, may have temporarily assumed hematopoietic functions. In five weeks, the organ was entirely normal, for both parenchyma and histiocytic components had returned to a characteristic organization.

#### CONCLUSIONS

A single large dose of diacetone alcohol administered to rats by stomach tube causes destruction of erythrocytes and reduction in the amount of hemoglobin. Both return to normal within a few days.

There are also cloudy swelling, vacuolization and granulation of the hepatic cells, reaching the maximal degree in twenty-four hours. This process ends in complete recovery. It is accompanied by early destruction and disappearance of the histiocytes, and is followed by late reappearance of these cells in vastly increased numbers with hematopoietic activity.

# MULTIPLE GANGLIONEUROMA

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The term "ganglioneuroma" has been applied to different kinds of tumors occurring either in the central or in the peripheral nervous system in which ganglion cells have been found. Just as in the spongioblastomas one may find all transitions from the most primitive spongioblast to adult fibrillary astrocytes, so in the ganglioneuromas one may find transitions from primitive neuroblasts to adult ganglion cells. In many ganglioneuromas occurring in the brain, admixture with neoblastic glial elements may be found. A comprehensive review of this subject was given by Wahl.<sup>1</sup> More recently a collection of ninety-three reports of cases from the literature was published by McFarland.<sup>2</sup> They gave evidence of the various locations in the body where such tumors occur. The present case is added because it seems to represent the only example, as far as we can determine, in which ganglioneuromas of benign type were found simultaneously in the cervical and abdominal regions, one in the region of the stellate ganglion, the others retroperitoneally.

A white woman, 23 years of age, was admitted to Passavant Hospital in the service of Dr. A. H. Curtis. Her complaints dated back over a period of one and one-half years. During this time she had lost 25 pounds (11.3 Kg.) in weight, had felt excessive fatigue and had noticed bilateral pressure in the inguinal region similar to menstrual pain. At the age of 4 years, she had had a lump removed from the left side of her neck, which was said to have been a "fatty nerve tumor." One month after its removal it reappeared and had been present until four weeks before, when it had been removed by Dr. Geza de Takats. It was situated deep in the anterior region of the neck, in the vicinity of the stellate ganglion. It was of the size of a child's fist and extended from the edge of the pleura to the transverse process of the fourth cervical vertebra.

Vaginal examination by Dr. Curtis revealed a small, freely movable uterus, displaced to the left, and an ill defined, relatively fixed, sarcoma-like mass, located

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From the Department of Surgery and the Institute of Neurology, Northwestern University Medical School.

1. Wahl, H. R.: J. M. Research **30**:205, 1914.

2. McFarland, J.: Arch. Path. **11**:118, 1931.

retroperitoneally in the right pelvis, which faded away as it entered the mid-abdomen. At operation, a chain of tumor masses, four or five in all, was found retroperitoneally to the right of the spine, extending from the kidney down into the pelvis. These masses were rubbery or gummatous in their consistency, relatively fixed, and apparently moderately vascular, and did not contain softened areas. The left kidney was about one-half normal size, and the left adrenal gland, which was from 3 to 4 times normal size, contained cystic areas.

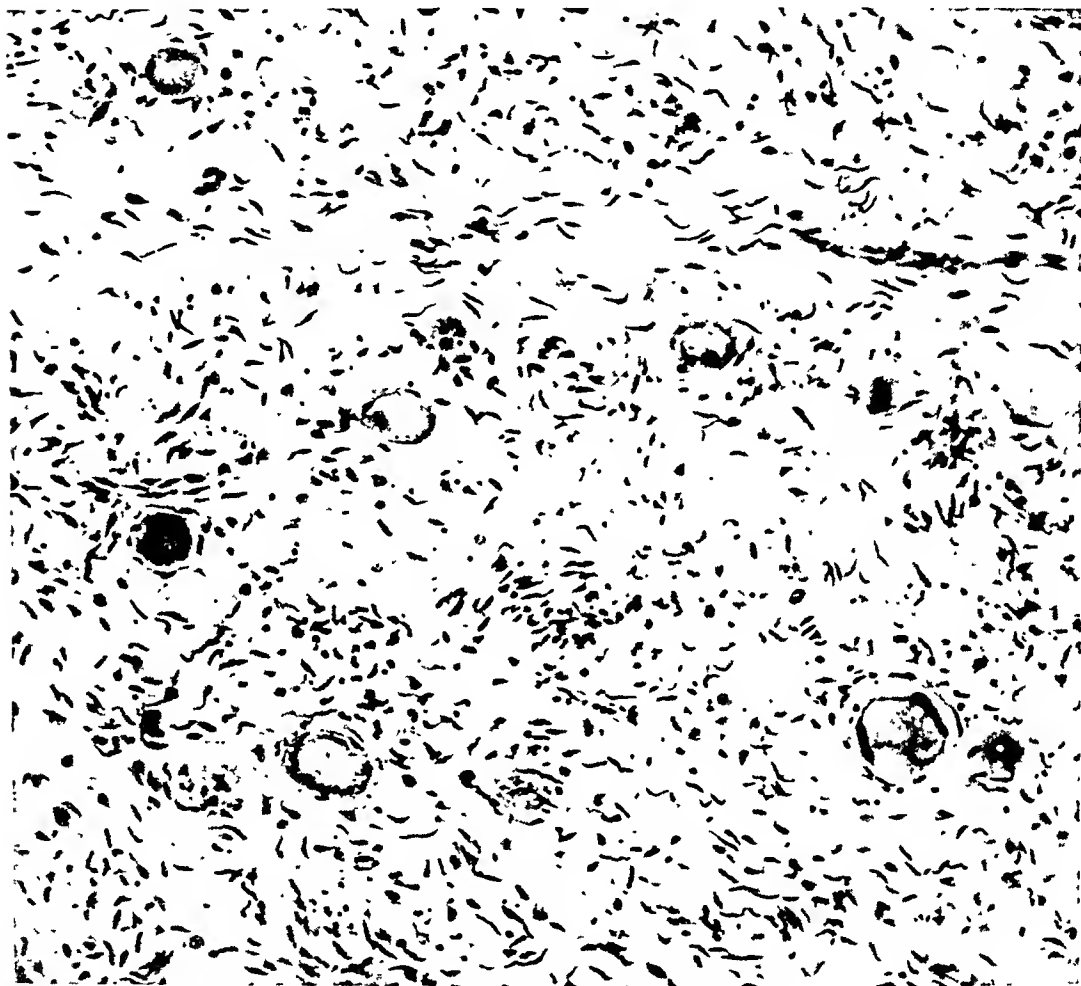


Fig. 1.—Section stained with cresyl violet; Leitz objective; ocular; 10  $\times$ . It demonstrates the general histologic appearance of the tumor. Ganglion cells of the type found in spinal ganglions are seen, with numerous spindle-shaped and oval nuclei in the rest of the tumor.

Both tumors were identical in their histologic structure. They contained ganglion cells similar to those found in spinal ganglions, which measured approximately 30 microns in diameter; each was surrounded by a fine capsule of connective tissue. They contained as a rule one, but on occasion two, large eccentric nuclei. Smaller, less well differentiated cells were infrequently seen. The main part of the tumor was formed by unmyelinated nerve fibers, which were covered



with a thin, fibrous sheath. In sections stained with cresyl violet, numerous spindle-shaped nuclei were seen, some of them assuming more oval forms. Fat tissue invaded the tumor masses, together with foci of lymphocytes.

This multiple occurrence of such an adult type of ganglioneuroma has its counterpart in the multiple neurinomas of von Recklinghausen's

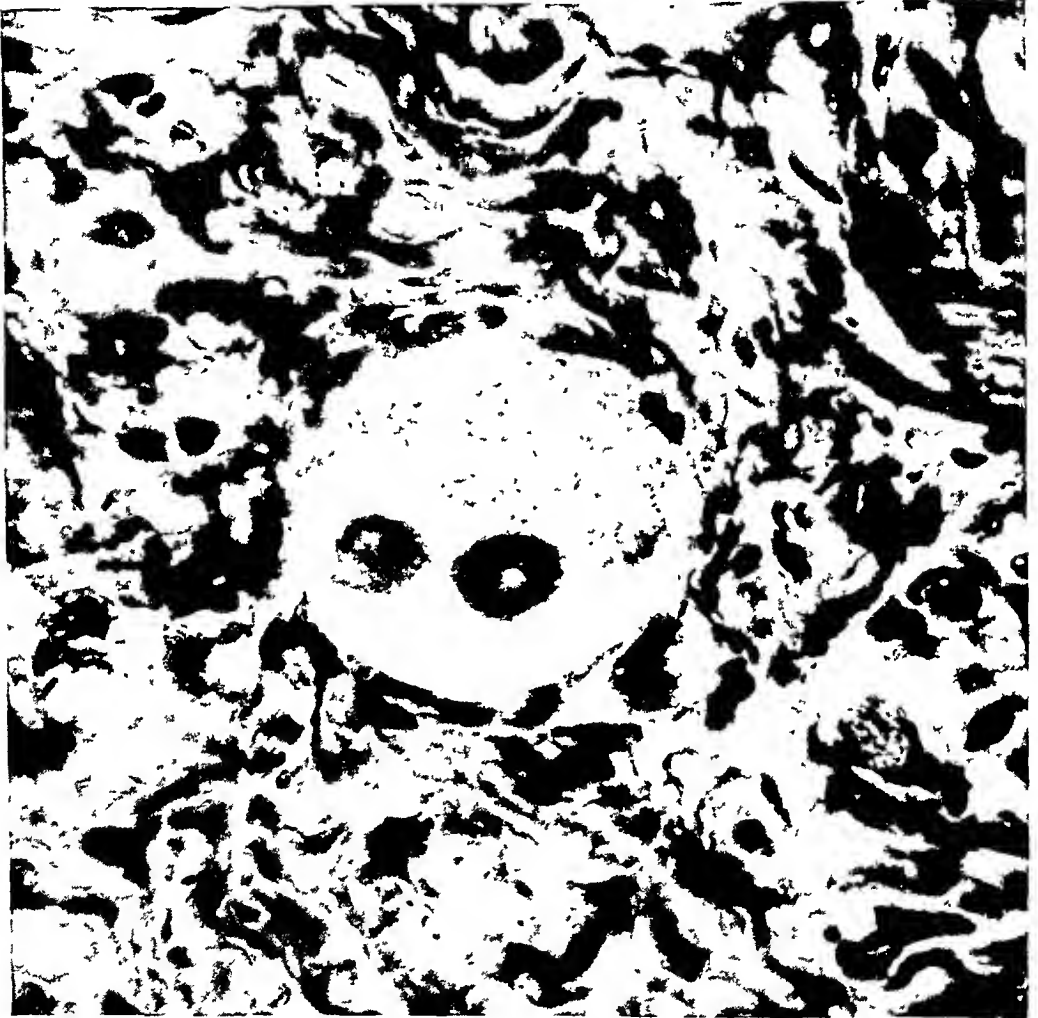


Fig. 2.—Davenport stain; Zeiss objective; 3 mm. apochromatic lens; ocular, 10  $\times$ . It demonstrates a binuclear ganglion cell with pericellular basket formation of unmyelinated nerve fibers, and unmyelinated nerve fibers in the environment.

disease. It is evident from the history that a neoplastic tendency was already present in the patient in early childhood.

#### SUMMARY

A case of multiple ganglioneuromas is presented, with simultaneous occurrence in the neck and the retroperitoneal region.



# HÜRTHLE CELL TUMOR

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Hürthle cell tumor of the thyroid gland is a proliferation of the cell described originally by Hürthle<sup>1</sup> in 1894 as a normal constituent of the thyroid gland, identical with the "parenchymatous" cells of Baber,<sup>2</sup> and as a cell capable of reproducing thyroid tissue when called on to do so. The tumor is an exceedingly rare one, only two references being found in the literature, one in Ewing's "Neoplastic Diseases"<sup>3</sup> (p. 953) where there is an illustration carrying the legend "Carcinoma of thyroid. Encapsulated acidophile cell adenocarcinoma. Hürthle cell tumor," and the other a recent article by Haagensen,<sup>4</sup> in which two cases are mentioned. The tumor may be either benign or malignant. The case we have to report is that of a benign growth, or so-called "Hürthle cell adenoma," of the thyroid gland.

## REPORT OF CASE<sup>5</sup>

A white woman, aged 54, was admitted to Sydenham Hospital in May, 1930, in the service of Dr. Louis Friedman, complaining of swelling in the neck and fainting spells. She had had a small goiter for years, which had become appreciably larger since January, 1930, following which she had had three "spells of unconsciousness," each lasting about one hour. She was told by those who had witnessed these "spells" that there was diffuse muscular twitching during the period of unconsciousness. Since January, 1930, there had been only slight loss of weight. Palpitation of the heart followed excitement or exertion. She was not visibly nervous, and did not perspire much. The menstrual and marital histories were normal.

The lateral lobes and the isthmus of the thyroid gland were moderately and uniformly enlarged. No nodules were noted. The veins of the neck were distended. There were no ophthalmic signs of Graves' disease, and no tremors of the

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From the Pathological Laboratories of Sydenham Hospital.

1. Hürthle, K.: Arch. f. d. ges. Physiol. **56**:1, 1894.

2. Baber, E. C.: Phil. Tr. Roy. Soc., London **209**:270, 1881.

3. Ewing, J.: Neoplastic Diseases, ed. 3, Philadelphia, W. B. Saunders Company, 1928, pp. 952 and 953.

4. Haagensen, C. D.: Am. J. Cancer **15**:2063, 1931.

5. Dr. Louis Friedman and Dr. William Stone placed this case at our disposal.

fingers. There was slight enlargement of the heart to the left and downward, with a blowing systolic murmur at the apex. There was marked pulsation of the abdominal aorta.

The basal metabolic rate (Sanborn-Benedict machine under optimal conditions) on May 8 was  $+40$ . With rest and compound solution of iodine, it was reduced by May 13 to  $+18$ . On May 15, under local anesthesia, three quarters of the right lobe of the thyroid gland was removed. The patient was discharged on May 24.

Ten months later the patient returned complaining of a peculiar sense of constriction about the head. There was marked enlargement of the thyroid gland from the left lobe past the midline. The swelling was solid, not tender, and firmly attached to the trachea. There were no subjective symptoms or physical signs of Graves' disease. The basal metabolic rate, however, was  $+41$ . On March 4, 1931, the left lobe was removed. Subsequently the basal rate dropped to  $+3$ , and when the patient was seen in July, it had dropped to  $-18$ . At that time, a large nodule was palpated at the site of the second operation, but this later disappeared.

Microscopic examination of the right lobe of the thyroid gland revealed a toxic, nonexophthalmic goiter. In the left lobe, microscopic section showed the tumor to consist of many small alveoli, long strands of palisade-like cells and, occasionally, syneytium-like masses. Some alveoli contained a small amount of colloid. The cells were large, fat-free cells, either polyhedral or irregularly shaped. They were clearly outlined. The cytoplasm was oxyphilic and finely granular. The nuclei were eccentrically placed and were small and vesicular, with nucleoli, and were rich in chromatin. No mitotic figures were seen, though a few double nuclei were noted. In addition, the picture of toxic, nonexophthalmic goiter was present, with many areas of lymphocytic infiltration. The diagnosis was "toxic nonexophthalmic goiter, and Hürthle cell adenoma of the thyroid gland." When the original slide was studied again, the same changes were recognized, in addition to the former picture (figs. 1 and 2).

The rarity of the growth and the apparent lack of literature concerning it have led us to seek more information, and to attempt to determine the exact nature of the tumor.

The Hürthle cell is a large, eosinophilic cell, rich in cytoplasm, found by Hürthle<sup>1</sup> on the outer surfaces of the walls of the follicles of the thyroid gland. He, as well as Zielinska,<sup>5a</sup> who had also seen these cells, believed them to be masses of immature cells concerned in the growth of the follicles. However, Mueller<sup>6</sup> and Michaud,<sup>7</sup> in describing the thyroid gland histologically, failed to find these cells, and Mueller stated that they were probably parts of the wall of the follicle cut obliquely. Maximow<sup>8</sup> also proved by careful dissection of normal and pathologic thyroid glands, and with wax-plate reconstructions, that these interfollicular cells do not exist. Boechat,<sup>9</sup> in his thesis on the thyroid gland, failed to mention any cells resembling these

5a. Zielinska, M.: *Virchows Arch. f. path. Anat.* **136**:170, 1894.

6. Mueller, L. R.: *Beitr. z. path. Anat. u. z. allg. Path.* **19**:127, 1896.

7. Michaud, L.: *Virchows Arch. f. path. Anat.* **191**:63, 1908.

8. Maximow, A.: *Text-Book of Histology*, Philadelphia, W. B. Saunders Company, 1930, p. 704.

9. Boechat, P. A.: *Thèse*, Paris, 1873, p. 12.

follicular cells. Horsley,<sup>10</sup> in investigating the embryonic tissue seen by Baber and by Hürthle in the thyroid gland, stated that in some animals a large quantity of this tissue could be found in a single mass beneath the capsule of the thyroid gland, but separately and distinctly encapsulated. He believed that the researches tended to refute the like-

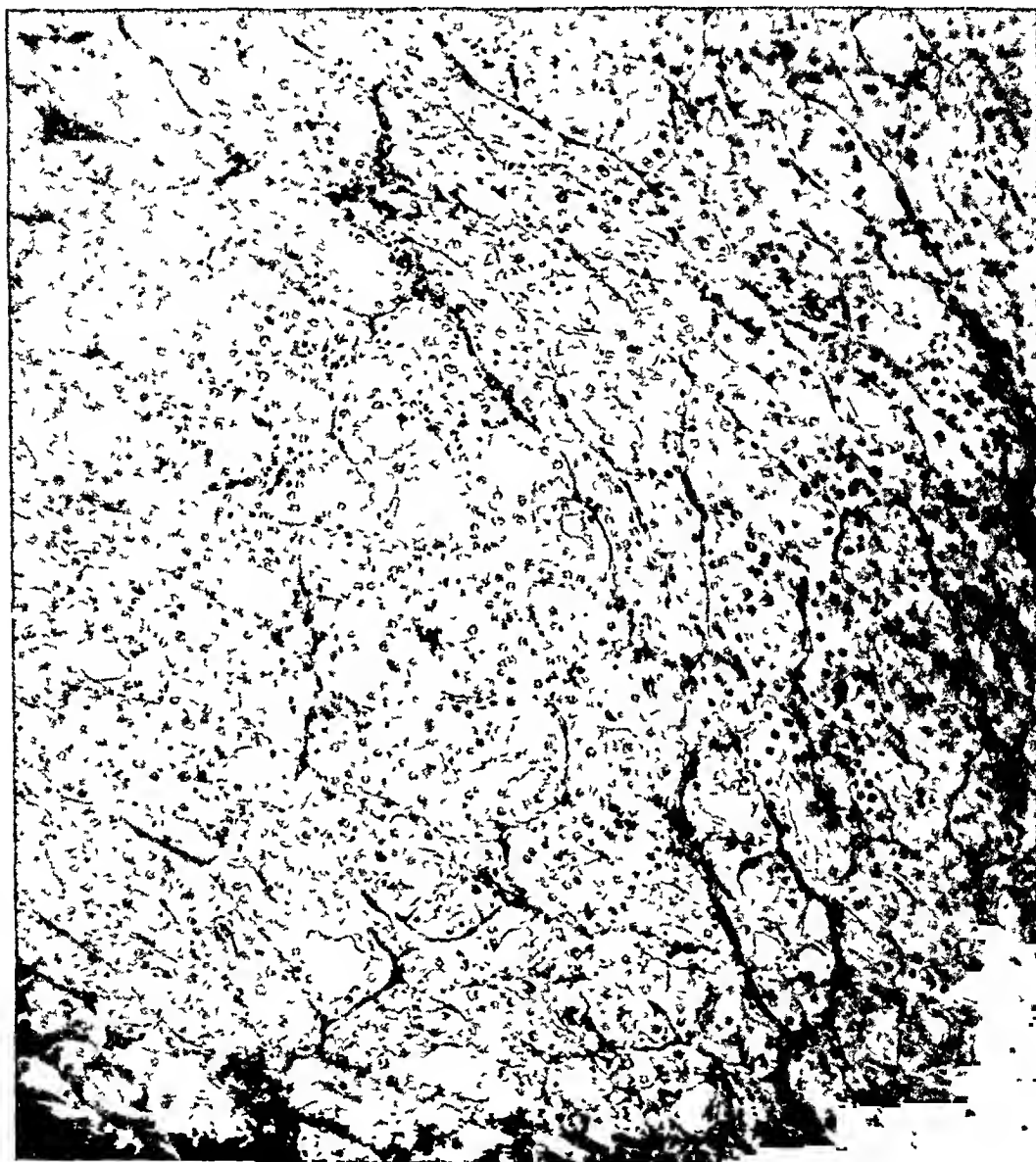


Fig. 1.—Hurthle cell adenoma under low power magnification.

lihood of this tissue ever developing into acini of the thyroid gland. Our conclusion from these facts is that the Hurthle cell is not always present in the parenchyma of the thyroid gland, though it sometimes may be, and that it therefore is not an integral part of that gland.

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10. Horsley, V.: *Lancet* 2:1163, 1886.

Welsh<sup>11</sup> in 1898, describing the parathyroid glands, referred to the masses of embryonic tissue seen in the thyroid gland by Hurthle and by Zielinska, and stated that they correspond sometimes to the internal, and sometimes to the external parathyroid glands. Erdheim<sup>12</sup> also referred to oxyphilic cells in the parathyroid glands that resembled the Hurthle cells. Sandstroem,<sup>13</sup> who was the first to describe the parathyroid glands, also thought that they had an embryonic function and would

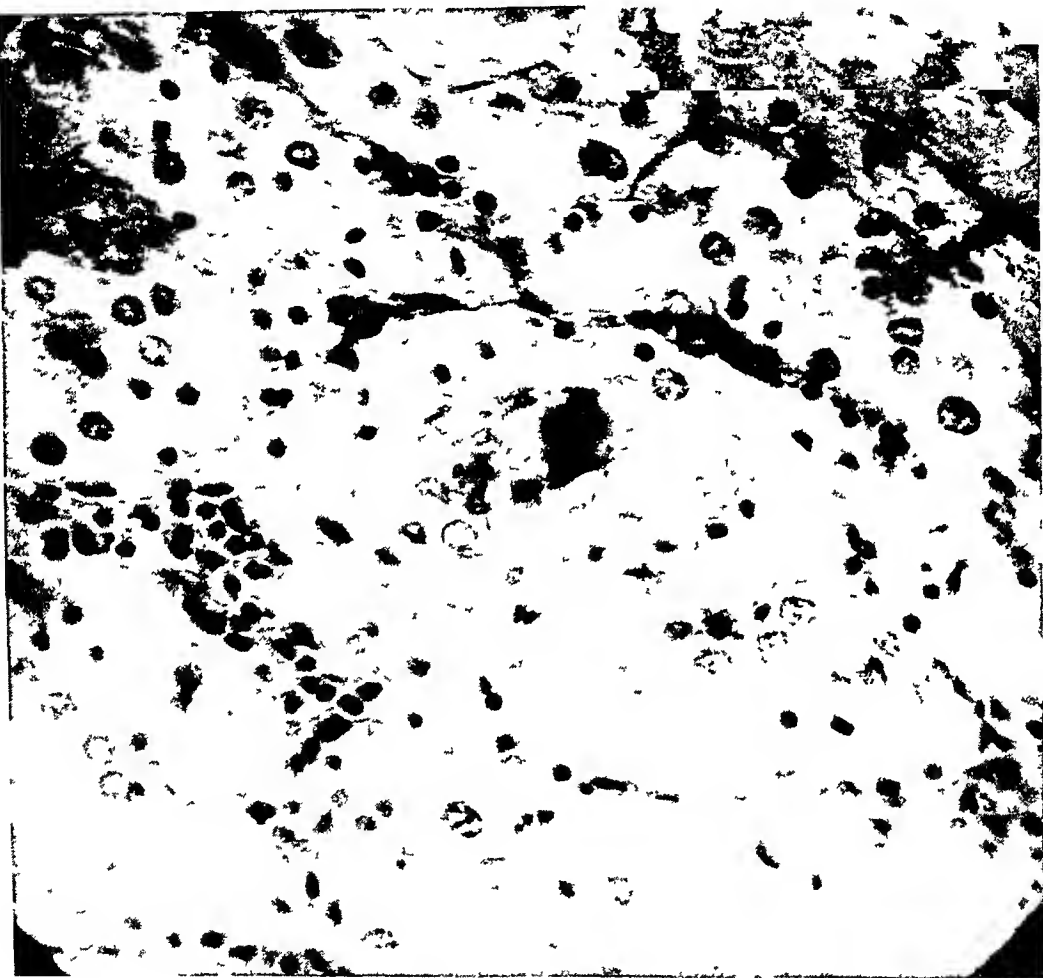


Fig 2—The same tumor as shown in figure 1, but under high power magnification. Note the large, polyhedral cells, "foamy" and granular, with nuclei placed eccentrically. Some are rich in chromatin, with many nucleoli. Some colloid-filled acini are seen. Note the resemblance to adrenal gland cells and hepatic cells.

form fresh thyroid tissue when required. The cells of the internal parathyroid glands described by Kohn<sup>14</sup> were obviously the same cells

11. Welsh, D. A. *J. Anat. & Physiol.* **32**:292, 1898.

12. Erdheim, J. *Beitr. z. path. Anat. u. z. allg. Path.* **33**:158, 1903.

13. Sandstroem, I. *Schmidt's Jahrb.* **5**:423, 1880.

14. Kohn, A. *Arch. f. mikr. Anat.* **44**:366, 1895.

that had been previously seen by Baber, Hürthle and Zielinska and thought by them to be interfollicular cells with an embryonic function.

The Hürthle cell therefore appears to be identical with the oxyphilic cell of Welsh, which is one of the two types of cells making up the parenchyma of the parathyroid gland. These cells have been described by Welsh<sup>15</sup> as being of two types, namely, chief (principal) cells and oxyphilic cells.

The former have a relatively small, clear cytoplasmic body with a relatively large, clear nucleus. The latter have a large, usually granular cytoplasmic body with a small, dark nucleus. The granules are highly oxyphilic. Later evidence tends to show that these two types of cells are identical, but represent different stages of activity, the oxyphilic cells showing the resting stage, and the principal cells the discharging stage. Erdheim<sup>16</sup> believed that these are all chief cells up to the tenth year, and Harnett<sup>17</sup> concurred in this opinion, because such cells do not begin to secrete until a person has reached the age of 10.

That these cells of the parathyroid glands should be found within the parenchyma of the thyroid gland is not surprising when one considers the anatomic and embryologic relationships of the two glands. A well known case of such ectopia is that of the presence of suprarenal gland rests in the kidney (Grawitz<sup>18</sup>). This similarity has already been noted by Berard and Alamartine.<sup>19</sup> Also there is the case reported by Kolodny<sup>20</sup> as one of hypernephroma of the thyroid gland, though he was unable to locate a primary tumor. This case Hendriock<sup>21</sup> considered as an example of a tumor of the thyroid gland definitely arising from a parathyroid gland rest.<sup>22</sup>

The parathyroid glands are by no means constant in their anatomic position. Most frequently there are four glands, the superior pair being situated near the lateral margin, and the inferior pair near the

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15. Welsh, D. A.: *J. Path. & Bact.* **5**:201, 1898.

16. Erdheim, J.: *Wien. klin. Wchnschr.* **41**:974, 1901.

17. Harnett, W. L.: *A Histological Study of the Human Parathyroid Gland*, 1907, Longmans, Greene & Co., London.

18. Grawitz, P.: *Virchows Arch. f. path. Anat.* **93**:39, 1883.

19. Berard, L., and Alamartine, H.: *Compt. rend. Soc. de biol.* **66**:619, 1909.

20. Kolodny, A.: *Arch. Path.* **1**:37, 1926.

21. Hendriock: *Centralbl. f. allg. Path. u. path. Anat.* **38**:385, 1926.

22. The resemblance of parathyroid gland cells to suprarenal gland cells has been noted before (Richardson, quoted by Geis: *Ann. Surg.* **47**:523, 1908), and they have also been found to resemble the carotid body cells (Prenant: *Cellule* **10**:18, 1894) and the hypophyseal cells (Welsh, footnote 15). This close resemblance between chromaffin cells in different glands has been further established by Koopman (Frankfurt. *Ztschr. f. Path.* **25**:342, 1921), who bases this resemblance on the acidophilia of the cells. The analogous occurrence of ectopic tumors of these glands may be more than just a coincidence.

median margin, of the lateral lobes of the thyroid gland. However, aberrant parathyroid glands are common (Marine<sup>23</sup>). They may be found on the anterior surface of the thyroid gland (Millzner<sup>24</sup>), at



Fig. 3.—Thyroid gland with parathyroid gland inclusions: (a) acini of thyroid gland; (b) parathyroid gland (Hurthle) cells; (c) diffuse lymphocytic infiltration.

23. Marine, D., in Tice, Frederick: *Practice of Medicine*, Hagerstown, Md., W. F. Prior Company, Inc., 1929, vol. 8, p. 264.

24. Millzner, R. J.: *J. A. M. A.* 88:1053, 1927.

the base of the tongue (Wood<sup>25</sup>), at the base of the thymus (Duperie,<sup>26</sup> Pepere<sup>27</sup>), in the hypophysis (Pepere<sup>27</sup>), within the thorax (Meyer<sup>28</sup>), and in the thyroid gland (Sandstroem,<sup>13</sup> Kohn,<sup>14</sup> Cristiani,<sup>29</sup> Gley,<sup>30</sup> Getzowa,<sup>31</sup> Welsh,<sup>11</sup> Wellbrock<sup>32</sup> and others). According to Wellbrock,<sup>32</sup> careful search in removed thyroid glands has shown eight per cent of them to contain aberrant parathyroid gland tissue. We have recently seen such a case (fig. 3).

The apparent cause of these so frequent aberrant positions of the glands lies in their embryologic relationships. The parathyroid glands develop from thickenings of the third and fourth branchial clefts on each side. The primordium on the third cleft is close to the bud of the thymus, while that on the fourth cleft is close to the lateral bud of the thyroid gland (Maximow<sup>33</sup>). A pair of diverticula arise from the fifth branchial pouch and form what are termed the ultimobranchial or postbranchial bodies (Getzowa<sup>31</sup>). These fuse with the thyroid gland, but contribute no true thyroid gland tissue (Gray<sup>34</sup>).

From the anatomic, histologic and embryologic data just recorded it is readily seen why parathyroid gland rests of oxyphilic cells occur within the thyroid gland, and also that the Hürthle cell is, in all probability, the oxyphilic or Welsh cell.

The fact that these inclusions of parathyroid gland cells can assume neoplastic characteristics is also established. True tumors of the parathyroid gland, however, are not common, most of the new growths being in reality hyperplasias associated with rickets, osteitis fibrosa, osteoporosis, nephritis, epilepsy and other conditions (Goedel,<sup>35</sup> Erdheim,<sup>36</sup> Kerl,<sup>37</sup> Gold,<sup>38</sup> Mandl,<sup>39</sup> MacCallum,<sup>40</sup> Wilder,<sup>41</sup> Thompson and

25. Wood, F. C.: Proc. New York Path. Soc. **16**:84, 1916.

26. Duperie, R.: Compt. rend. Soc. de biol. **99**:324, 1928.

27. Pepere, A.: Arch. de méd. expér. et d'anat. path. **20**:21, 1908.

28. Meyer, A. W.: Anat. Rec. **3**:272, 1909.

29. Cristiani, H.: Arch. de physiol. norm. et path. **5**:279, 1893; J. de physiol. et de path. gén. **7**:261, 1905.

30. Gley, E. A.: Arch. physiol. norm. et path. **24**:81, 1892; Compt. rend. Soc. de biol. **4**:18 and 46, 1897; Compt. rend. XII Cong. Internat. Med., Moscow **3**:190, 1897; Presse méd. **6**:17, 1898.

31. Getzowa, S.: Virchows Arch. f. path. Anat. **188**:181, 1907.

32. Wellbrock, W. L. A.: J. A. M. A. **92**:1821, 1929.

33. Maximow (footnote 8, p. 700).

34. Gray, H.: Anatomy, Philadelphia, Lea & Febiger, 1924, p. 1282.

35. Goedel, A.: Wien. klin. Wchnschr. **38**:246, 1925.

36. Erdheim, J.: Ztschr. f. Heilk. **25**:1, 1904.

37. Kerl, W.: Deutsche med. Wchnschr. **21**:127, 1925.

38. Gold, H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **41**:63, 1928.

39. Mandl, F.: Arch. f. klin. Chir. **143**:245, 1926.

40. MacCallum, W. G.: Bull. Johns Hopkins Hosp. **16**:87, 1905.

41. Wilder, R. M.: Endocrinology **13**:231, 1929.

Harris,<sup>42</sup> Hunter,<sup>43</sup> Herxheimer,<sup>44</sup> Schmorl,<sup>45</sup> Todyo<sup>46</sup> and Parreiro and Casto Freire<sup>47</sup>). These changes may be primary, leading to the conditions mentioned (Mandl<sup>48</sup>), or they may be secondary to those conditions (Boyd, Milgram and Stearns<sup>49</sup>).

As is well known, true tumors of the parathyroid gland can be either benign or malignant. These tumors have been found both external to and within the thyroid gland.

The parathyroid gland tumors within the thyroid gland were described by Langhans,<sup>50</sup> who classified the epithelial tumors of the thyroid gland as follows: (1) proliferating struma, (2) carcinomatous struma, (3) metastasizing struma, (4) parastruma (parathyroid or glycogen-containing struma of Kocher), (5) small alveolar, large cell struma (probably struma postbranchialis of Getzowa), (6) papilloma and (7) cancrroid.

In describing the fifth group, Langhans stated that the cells of the alveoli of the struma can be compared with hepatic cells or fat-free cells of the adrenal glands. The cells are very large, from 15 to 30 microns in diameter, polyhedral and rich in cytoplasm, which is acidophilic. They contain no lipoids. The nucleus is often eccentric, measures from 6 to 8 microns in diameter, and is round and vesicular, with its membrane folded and wrinkled; it contains numerous chromatin granules and threads, with a small eosin-red nucleolus.

When Getzowa<sup>51</sup> described struma postbranchialis, she classified the cells into four types: (1) water-clear cells, (2) rose-red cells, (3) oxyphilic cells of Welsh and (4) syncytium-like cells.

The tumor corresponding to the small alveolar, large cell struma of Langhans and to the oxyphilic cell tumor of Getzowa is the tumor seen by us. It is described by Ewing<sup>3</sup> as consisting of "relatively small, well-formed alveoli lined by one or more layers of very irregular cells. Some are clear, cuboidal or cylindrical, or irregularly polyhedral. Others are large, sometimes of giant size, finely granular, eosinophile and opaque, resembling granular suprarenal or liver-cells. The nuclei are small, vesicular, with visible nucleoli." Having seen two such tumors, Ewing suggested that these oxyphilic cells may represent hypertrophic Hürthle cells, and thus called the growth Hürthle cell tumor.

42. Thompson, R. L., and Harris, D. L.: *J. M. Research* **19**:135, 1908.

43. Hunter: *Proc. Roy. Soc. Med.* **23**:27, 1928.

44. Herxheimer, G., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 8, p. 548.

45. Schmorl, G.: *Ergebn. d. inn. Med. u. Kinderh.* **4**:403, 1909.

46. Todyo, R.: *Frankfurt. Ztschr. f. Path.* **10**:219, 1912.

47. Parreiro and Casto Freire: *Compt. rend. Soc. de biol.* **95**:1590, 1892.

48. Mandl, F.: *Zentralbl. f. Chir.* **56**:1739, 1929.

49. Boyd, J. D.; Milgram, J. E., and Stearns, G.: *J. A. M. A.* **93**:684, 1929.

50. Langhans, T.: *Virchows Arch. f. path. Anat.* **189**:69, 1907.

51. Getzowa, S.: *Virchows Arch. f. path. Anat.* **205**:208, 1911.



## CONCLUSIONS

The Hürthle cell, as an integral part of the thyroid gland, does not exist, because it is not always present in the gland, it is identical with the parathyroid gland oxyphilic cells of Welsh, and it takes its embryologic origin in close relationship with the parathyroid gland.

Hürthle cell tumor of the thyroid gland is a misnomer because it leads to the erroneous impression that it originates from the tissue of the thyroid gland.

The tumor really originates from parathyroid gland rests within the thyroid gland, and is an example of parastruma as described by Langhans and by Getzowa; in its relationship to the thyroid gland, it corresponds to renal hypernephroma.

The symptoms of convulsions and constriction exhibited by our patient suggest increased secretion of the parathyroid glands.

# RADIATE FORMATION DUE TO A HYPHOMYCETE (ASPERGILLUS?)

AS SEEN IN PULMONARY GRANULOMATOSIS OF A CAPYBARA  
(HYDROCHOERUS HYDROCHOERUS); THE NATURE OF  
FUNGUS INCRUSTMENTS IN GENERAL

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That a moisture-loving and aerobic plant like a mold should be found parasitic in the lungs of such an animal as the capybara can occasion no surprise to the sophisticated pathologist; however, he may still be curious as to the rôle of the fungus as a pathogen and the part that the animal might play as a porter of analogous human fungous disease. But in addition to these considerations, which belong in the field of comparative pathology, three sidelights have developed that have immediate bearing either on human medicine or on the biology of fungus: (1) the formation of radiate fungi in disease other than actinomycosis; (2) a new, unique morphology for radiate fungi (except in experimental rabbits); (3) the nature (genesis) of the hyaloid incrustations<sup>1</sup> occasionally observed on other pathogenic fungus cells, such as *Coccidioides immitis* and *Actinomyces bovis*. This feature of incrustation, indeed, is, in the final analysis, the determining factor for the radiate fungus effect both in this capybara and in true actinomycosis; hence it merits special attention in its essentially mycologic and biologic aspects. The incrustation on the fungus of the capybara was particularly welcome for study in this connection because it affected a cell intermediate in size between the taxonomically widely separated fungi, *Coccidioides* and *Actinomyces*, constituting a stepping-stone that makes it simpler to compare the biologic processes concerned in the formation for all three, as well as for others.

## HISTORY OF CASE

The capybara was a full-grown female, dying Dec. 19, 1930, in captivity in the Philadelphia Zoological Garden. This animal is a rodent closely related to the

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1. This term will be employed in this paper in the loose, broad sense, i. e., simply of matter deposited on the exterior, without connoting its composition or source (internal or external).

guinea-pig (belonging to the Caviidae) and has a geographic range extending from Central to South America, i. e., from the Guianas to the river Plata in Argentina. It favors the banks of rivers and is semiaquatic in habits. During its seven months' stay in the local zoological garden, this particular animal had had no cage mates. However, its enclosure was in series with ones occupied by aquatic or semiaquatic birds (liable to and therefore potential reservoirs of aspergillosis); a small stream ran through the entire series of enclosures.

However, fungous disease was not encountered once in the fourteen capybaras on which autopsies had been done previously in the same zoological garden, and five of these had been in captivity for a longer period of time than this one (eight months, nine months, nine months, four years and eight years, respectively). The only birds upstream to the capybara had been penguins and ducks; but, significantly, a large group of the former had died more than fifteen months previously with extensive, more or less generalized aspergillosis. The ducks that were subsequently parked in the same enclosure after its disinfection, which are also liable to aspergillosis, but to a lesser degree, have not contracted aspergillosis to date. These factors of birds and *Aspergillus* are in point of view of da Fonseca's hints about the identity of the fungus found in the capybara. Further mention of this will be made in later paragraphs. This accumulation of evidence is thus conflicting as to the source of the infection, whether avian or related to inanimate environment, including food.

In conformity with the usual experience with wild animals, no symptoms of disease had been observed in this capybara even immediately prior to death. Dr. H. L. Ratcliffe, assistant pathologist to the garden, performed the autopsy and made the diagnosis histologically, and then referred the lungs to me for more detailed study. Since all of the tissues had been fixed in formaldehyde, cultural studies were precluded.

*Necropsy.*—The animal was not well nourished; fat was scanty, and the muscles were thin and pale. The skin was scaly and dry.

Internally, the outstanding finding was a perforated duodenal ulcer, with secondary serofibrinous peritonitis. The ulcer was 1 cm. in diameter and had the conventional punched-out characteristics of the lesion in man. The material around it externally was fibrinous rather than fibrous. The peritoneal cavity contained 100 cc. of bloody fluid, intermingled with flakes of pus.

There were some thin, fibrous adhesions between the cecum, duodenum and gallbladder. Except for some swelling and pallor, the kidneys appeared normal. In the spleen, the follicles were large and prominent; the pulp was bright red-brown. The lymph nodes in all regions were large, firm and pale.

The lungs, exclusively the seat of the mycotic findings in the case, were voluminous, considerably congested and edematous, and exhibited the barest suggestion of lobular consolidation, uniformly distributed through both lungs. In addition, myriads of nodules, both subpleural and interstitial, were present. The subpleural ones resembled miliary tubercles, but were perhaps paler, more translucent and homogeneous, suggesting for the moment multiple metastatic cancer. This effect was lost on the cut surface, however, where the appearance was like that of multiple, disseminated miliary tuberculosis. A predilection for peribronchial positions could not be made out. There were no cavities, sinus formations or other foci in keeping with actinomycosis of man.

*Histologic Examination.*—Histologic sections were cut from the following organs: heart, lungs, liver, kidney, spleen, pancreas, adrenal gland and thyroid gland. Except in the lung, no frank and significant abnormalities were recognized

in these organs. In the kidneys there was degeneration of the parenchymal cells, with thrombi in the glomerular tufts; acute degenerative nephritis was diagnosed. A moderate grade of cardiac degeneration was indicated by a dimming of transverse striations. The centers of the hepatic lobules were degenerate, and the periportal regions somewhat fibrosed. Otherwise the search for disease was without result.

Except for a few short stretches where the pleura was edematous and lightly infiltrated by lymphocytes, it could be regarded as normal. There was no excess

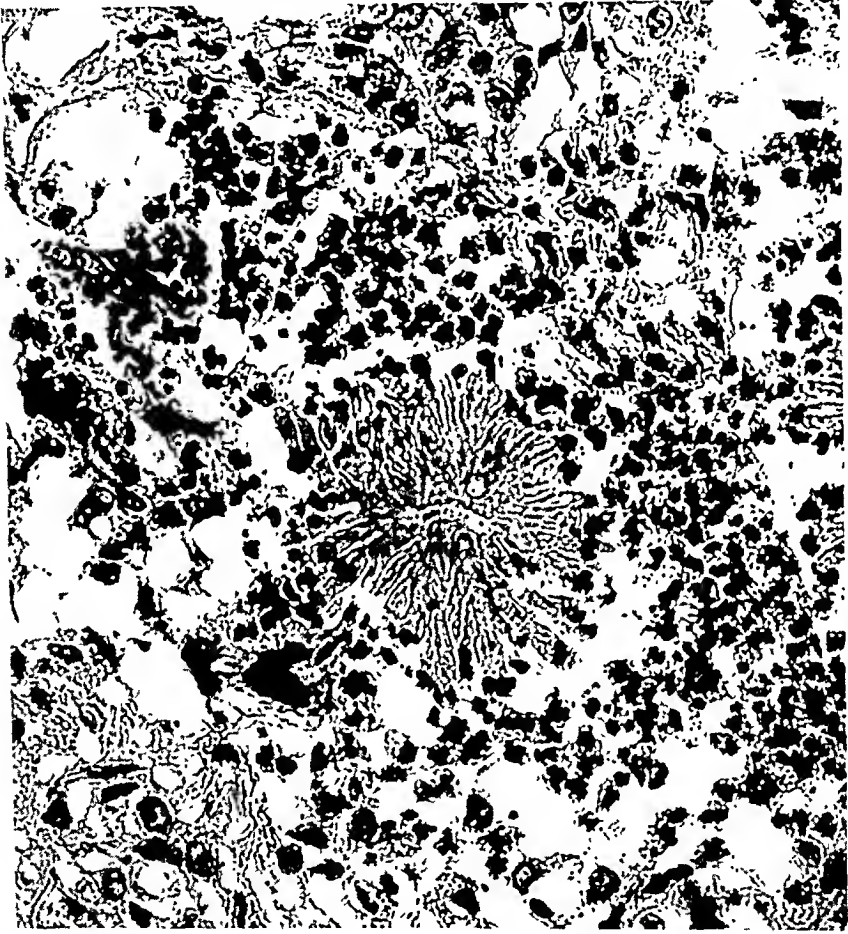


Fig. 1.—Hematoxylin and eosin preparation. A short stretch of hypha is included in the center of a radiate fungus; there is an infiltration by polymorphonuclears immediately around it, as in actinomycosis.

of fibrous tissue in the general pulmonary framework. The state of the air sacs varied widely from place to place; in but small part were they overdistended; for the most part, they appeared smaller than normal. In certain comparatively large areas, they were more or less solidly filled with a pink, finely granular precipitate of edema, red blood cells and perhaps desquamated epithelium. Fibrin, if present at all, was scanty. Phagocytes loaded with black pigment were numerous.

Granulomas: The granulomas comprised the most important feature. They were sometimes single and at other times were so closely placed as to produce comparatively large areas of consolidation. In parts, the solidifying substance was

the edema and epithelial desquamation (bronchopneumonic type of exudate) mentioned. At other times, polymorphonuclears were richly intermixed; when the latter was the case, they were generally close to the fungus organisms, which will be described later. Indeed, at times the granulomas appeared to consist almost exclusively of a group of polymorphonuclears clustered against one of these fungus organisms; however, on careful search some larger, rather granular and apparently disintegrating cells akin to endothelioid cells could generally be discerned in the periphery of the leukocytic accumulations. In larger granulomas there were increased numbers of these degenerate and more or less fused endothelioid cells.



Fig. 2.—A radiate fungus within a bronchial terminus (atrium). The iron hematoxylin preparation brings out lateral offshoots from one of the rays. The latter is not a common occurrence.

They differed from those commonly seen in tuberculosis in that whereas they had extremely broad and definite cytoplasm (often stellate in form) they were still indefinitely outlined. Indeed, the individual cells were with difficulty distinguishable from degenerate lining epithelium of the air sacs, and could, indeed, well be this. Giant cells were numerous; for the most part, they were of foreign body type. They frequently contained small portions of fungus rays. In a number of instances it was possible to make out (apparently isolated) giant cells that contained fragments of fungus, which deserve special mention because they occurred clearly within air spaces.

All of the larger bronchi were greatly distended but empty, while some of the smaller ones were completely occluded with more or less degenerated polymorphonuclears, and on search giant cells and fragments of the rays of the fungus could finally be located here also. It could be made out that there was a particular concentration of the pneumonic foci around the bronchi; but, in addition, numerous granulomas and other inflammatory effects were discoverable as far out as the subpleural parenchyma.

#### THE RADIATE FUNGUS

As remarked, the radiate fungus was almost invariably surrounded by more or less disintegrated polymorphonuclears. In point of size and conspicuousness, in point of radiation effect, and in point of the dense hyaloid composition of the rays, this organism was altogether comparable to *Actinomyces bovis*. On the other hand, the respects in which these radiate forms in the capybara differed from the conventional ones of human actinomycosis were as follows:

1. They did not exhibit the large size possible and known for actinomycosis. This could, however, be simply an expression of youth incident to the acuteness of this particular infection, the organisms not having had time to attain their fullest dimensions.

2. The organismal mass was uniformly spherical or elongated; that is, it was not lobulated as is so often the case in true actinomycosis. Again, this might be an expression of youth, but I believe that the elongated form is one to be expected—contingent on the elongated hyphae subsequently demonstrated in *in toto* preparations.

3. The radiate extensions of a mass were not club-shaped, as with actinomyces, but were more or less cylindric. However, they were of about the same length and width as those of *Actinomyces bovis* and were of the same hyaloid texture. The tips were not rounded conformable to their being part of a club, but appeared broken off short; or, the tips might have a more or less definitely angular termination (gladiolar).

4. As an outstanding difference, the center of the radiating mass was not granular (more or less disintegrated fungus substance); instead the outlines of a double-contoured cell could almost always be made out. It is emphasized that this cell had only superficial resemblance to a blastomycete; it did not bud, and its outlines were not the beautiful, geometrically circular ones of a blastomycete, but were comparatively uneven as though it had felt the effect of some shrinking agent (fixative?) and had become distorted. While this asymmetry might well be regarded as a function of the size of the cell, averaging as it does well above that of Gilchrist's organism and most of the other pathogenic yeastlike forms, I doubt it, because this irregularity of form is compatible with cells that are part of the hyphal structures subsequently

demonstrated in *in toto* preparations. Furthermore, after search, elongated examples of these double-contoured structures were met, and even suggestions that they were branching.

As with *Actinomyces bovis*, the relationship of the external hyaloid (radiate) material to the central cell is interesting; that is, in regard to whether this material is a product of the cell or a contribution from the inflammatory processes roundabout. In any event, the hyaloid material comprising the ray extended into direct contact with the cell in the interior of the fungus. Indeed, conditions were sometimes so favorable that the substance could be observed becoming integral with the fungicellulose in the wall of the micro-organism itself. I feel very strongly that this material is fungus in production and not host tissue.

5. Yet another difference consisted in the (only occasional) presence of striations passing obliquely (laterally and centrifugally) from both sides of the axis of a given ray. This imparted a feather or herring bone appearance to the ray, as though the striations were extending out from an invisible midrib.

*Gram Stain.*—The gram stain was of advantage only in that portions of many, but not all, of the rays stained gram-positive (varying irregularly in color from greenish blue to purple), thus serving to identify the location of the fungus organisms more promptly during searches under low power magnification. No filamentous structures were identified, such as are seen in *Actinomyces bovis*, by this technic. As usual, the metachromatic granules in the interior of the central cells were gram-positive.

*Giemsa Stain.*<sup>2</sup>—Likewise, no filamentous structures were brought out by the Giemsa stain, but the outlines of the fungus organisms were sharper, as usual, than by any other staining method. The rays stained more deeply (blue) than by other stains, but their substance showed no superior or additional differentiation.

*Stain for Bacteria.*—As a test for the presence of *Bacterium actinomycetum-comitans* or other such forms, a frozen section of lung (formaldehyde-fixed) was stained in weak basic fuchsin and examined without being dried or cleared (an approach to supravital methods). No bacteria were demonstrated.

*Haidenhain's Iron Hematoxylin.*—This regressive method stained the hyaloid substance of the rays of the fungus black. As usual, it was particularly useful for bringing out details, such as the plane of junction between the radiate extensions and the wall of the cell. The wall of the cell took the red counterstain. Again, the hyaloid incrustation was found to come into most intimate contact with the wall of the cell.

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2. Including the May-Grünwald modification.

However, the two did not blend; at least, in some cases the wall of the cell was recognizable as a pink, double-contoured shell independent of the rays.

*Polarized Light.*—None of the fungus was anisotropic in sections; this also applies to the spinulous processes on the rays.

*Fungus in in Toto Preparations.*—In order to examine further the branching of the fungus, which could not be satisfactorily determined in paraffin sections, and to get an idea of the form of the fungus *in toto*, pulmonary substance containing granulomas was softened and cleared

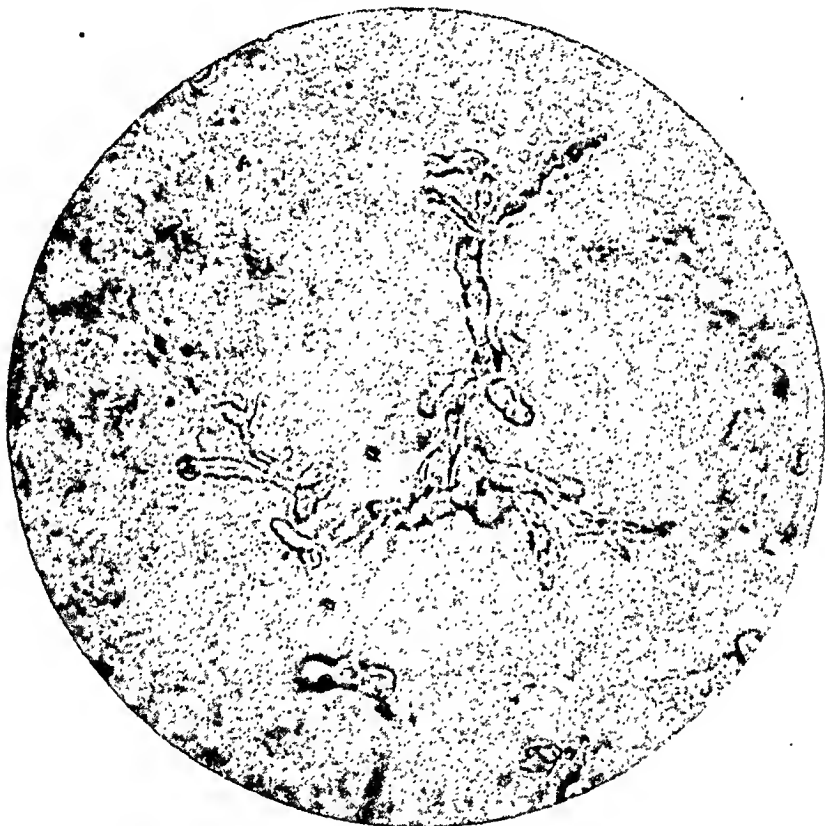


Fig. 3.—Mycelium in the center of a radiate fungus from pulmonary substance leached in potassium hydroxide solution.

by leeching in 10 per cent potassium hydroxide for several days. From time to time during this period, the material was shaken in order to facilitate softening; as the tissue had been previously fixed in formaldehyde, the pulmonary substance did not become unduly disintegrated. In the sediment of this preparation, the outlines of the rays had largely disappeared or were at most only suggested; in their stead there was a finely granular or homogeneous matrix, within which the mycelium was still splendidly preserved.

The hyphae were both simple and complex. Simpler ones consisted of a stretch of perhaps only three or four segments of fungus;



in the more complicated ones there would be three or four major branches and five or six short ones extending from the main, parent stem. The segments were broad, estimated at 4 microns in diameter, and two or three times as long. There was a definite, thick shell of fungicellulose, and in the interior a number of fine granules with two or three coarser ones. While most of the segments were cylindric, many were deformed or, what was rather striking, narrower at the proximal end than at the distal, giving a more or less trumpet shape. The terminals of some of the hyphae were bluntly bifid, recalling the form sometimes assumed by *Achorion schoenleinii*.

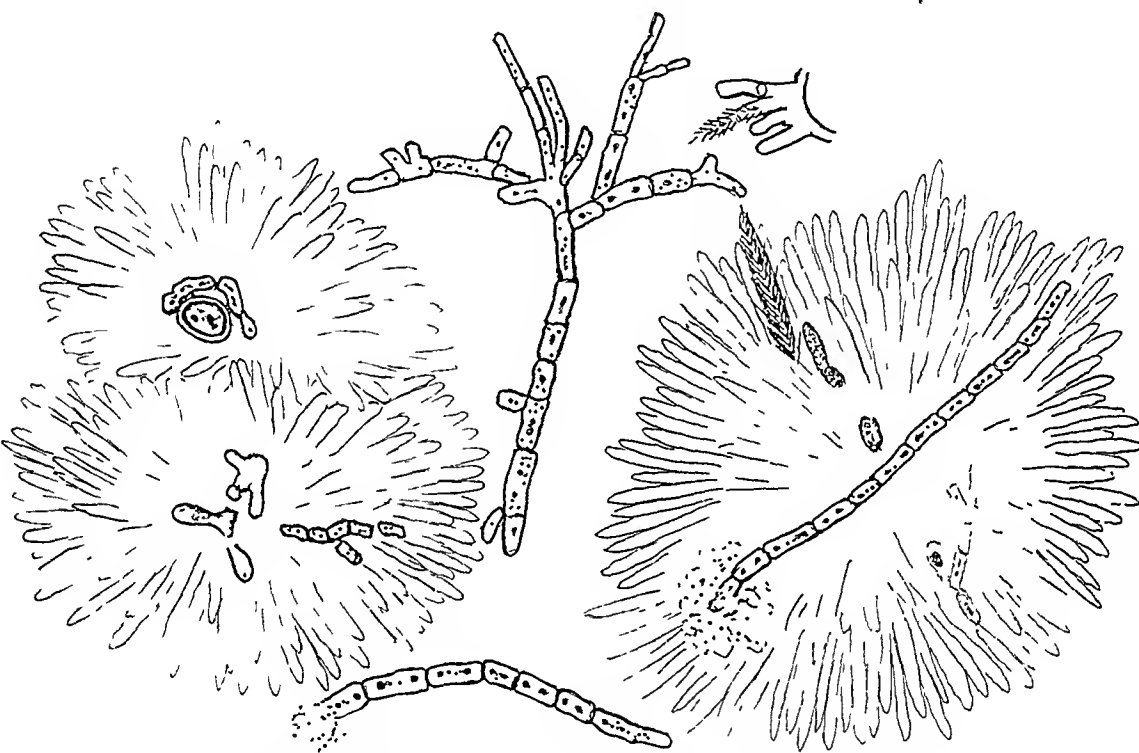


Fig. 4.—Radiate fungi leached by means of strong alkali from pulmonary tissue. The radiations were smooth, hyaloid and faintly yellowish green. They persisted around some of the hyphae; it appears that they had been dissolved from others.

#### SOURCE OF THE INFECTION

The morbid anatomy (no important fibroses) seems to indicate that this infection was not far enough developed to have been contracted in the wild; the animal had been in captivity for at least seven months. My own original suspicions that the organism might be an aspergillus, contracted from waterfowl nearby, are suggestively (and independently) supported by Dr. Olympio da Fonseca:<sup>3</sup> "As far as my experience

3. Dr. Olympio da Fonseca, of the Oswaldo Cruz Institute in Rio de Janeiro, exchanged sections of Brazilian chromoblastomycosis for mine from the capybara.

goes," he wrote, "the thing I saw most like to that which your capybara exhibits, was obtained by intravenous inoculations of rabbits with some strains of *Aspergillus fumigatus*. In such cases I saw quite often, around the injected spores, club-like structures not so developed as in your capybara, but quite conspicuous. I do not think one can reach any definite conclusion in this case, as no cultures are available."

As indicated in the history of the case, all data are so circumstantial that the source of the infection must be regarded as undetermined, although the data are suggestive (avian).

#### IDENTITY OF THE PARASITE

The coarseness of the metachromatic granules in the interior of the cells, with at the same time the comparatively heavy and broad walls, is not unlike what is seen in *Madurella*, but the black pigmentation was lacking. No other specializations were discovered in this material—no chlamydospores, no arthrospores, not to say asci and other such extremely high forms. So far as the form of the fungus in this tissue is concerned, the organism could be classified no farther than as a member of the *Arthrosporinae*. This is not an unusual experience for even the most complicated species of fungi as they infect animal tissue; in the latter, comparatively unfavorable environment, they do not tend to produce the more highly specialized examples of reproductive bodies, but tend only to vegetate or at best to reproduce only by the formation of comparatively nondistinctive, endogenous spores known as arthrospores.

The literature on actinomycetoid forms fortunately comes to one's assistance at this point and supplies near-duplicates of the fungus of the capybara, but only in experimental<sup>4</sup> animals. Thus, in kidneys of rabbits, Macaigne and Nicaud<sup>5</sup> secured forms that appeared to be identical with it save that their radiate extensions were more genuinely club-shaped. They were remiss in descriptions of the mycelium itself, and the illustrations are poor. However, the latter are satisfying as to the general radiate form and construction of the fungus, and the spaces in the center are comparable in location and size to those of the mycelium in the capybara. If allowance is made for the larger size of the fungi studied in the rabbit (therefore probably older), it becomes easier to reconcile the difference in shape of the clubs. All things considered, I feel that the two forms may be regarded as identical

4. This is fortunate, because there is no doubt as to the identity of the causative micro-organism.

5. Macaigne, M., and Nicaud, P.: *Bull. et mém. Soc. méd. d. hôp. de Paris* 52:551, 1928.

if it is recalled how labile the morphologic aspects of the fungi are, and that an aspergillus (the French workers used a pure culture of *Aspergillus fumigatus*) was the etiologic factor in the capybara.

*Oidium albicans*, however, has to be eliminated in view of the radiate form of fungus observed in the kidney in experimental rabbits by Magrou<sup>6</sup> (see fig. 14). Here, again, the morphology of the mycelium in the center was not furnished in detail, but it was fully as broad as that of the capybara. The winding course described by the author may not deserve emphasis, but certainly that of the mycelium in the capybara was not winding. Furthermore, clubs were but scatteringly distributed over the exterior of this radiate form of *Oidiums*. All other radiate forms of fungus or "grains" (see later paragraphs) were so widely discrepant as to be readily excluded from consideration with the ones of the capybara, mainly because their centers did not exhibit *wide* mycelium. Only *Madurella* exhibited such mycelium, but the "grains" of the disease caused by this organism are lacking in peripheral clubs and do not enter into the problem of differential diagnosis.

Incidentally, if it can be assumed that the capybara presented aspergillosis, the survey of the literature makes it appear that this experience is unique (1) in the occurrence of radiate forms of fungus apart from experimental animals (spontaneous) and (2) in the presence of aspergillotic granulomas in the lungs. (Heretofore the pulmonary lesions have been diffuse and devoid of radiate forms. Radiate forms were found only in the kidneys.)

#### THE NAME OF THE DISEASE

*Actinomycosis*.—Certainly, this disease cannot be admitted as actinomycosis. I am not in sympathy with the policy of assigning a newly discovered disease to a previously denominated infectious disease group that has an established etiology, unless the name of the latter disease connotes also the causative organism of the new disease.<sup>7</sup> If, theoretically, it were possible to reconcile the fungus in the capybara with the already established genus *Actinomyces*, of course the name actinomycosis would be proper, but this cannot be done, because the streptothrix of *Actinomyces* and the broad hyphae of the fungus found in the capybara indicate widely separated taxonomic positions.

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6. Magrou, J.: *Ann. Inst. Pasteur* **33**:344, 1919.

7. Nicaud, for instance, while disputing "aspergillar pseudotuberculosis" as the title of the disease in his (aspergillotic) experimental animals, did so on the basis of morbid tissue discrepancies rather than differences in etiologic agent (*Compt. rend. Soc. de biol.* **99**:1564, 1928).

To crystallize thought, it is in order to compare the disease of the capybara and actinomycosis. First, they have in common the same fundamental reactions of the tissues. Leukocytes are massed against and directly in contact with the micro-organism, and a low grade of chronic inflammation appears immediately peripheral to this. This is a form of reaction common to a number of other infections with fungi (true actinomycosis, coccidioidal granuloma, blastomycosis). It is true that from the gross standpoint the disease of the capybara was not outstandingly suppurative. Whether it could become so in the more advanced stages cannot be predicated at present. By analogy with chromoblastomycosis, it would not be essentially suppurative, but here analogy fails one, for by analogy with actinomycosis it would be suppurative.

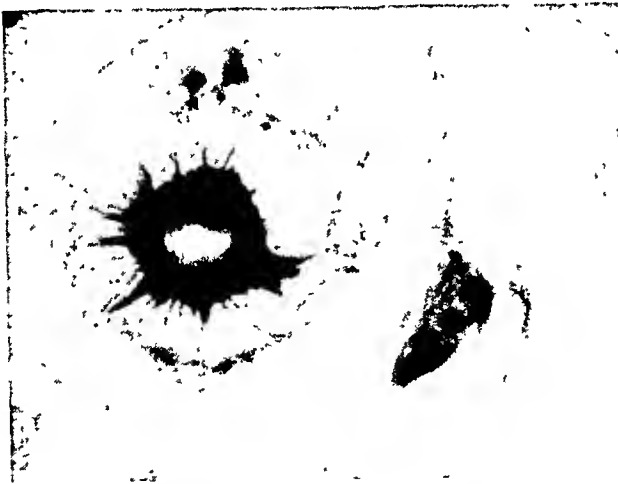


Fig. 5.—*Torula histolytica* in brain. Radiate extensions from the cell membrane pass outward into the thick, mucinoid envelop, bespeaking the homogeneity of the two. Dr. Walter Freeman supplied the photomicrograph.

Second, in both diseases the causative organism has the capacity to develop hyaloid incrustations that send out such processes as to impart a generally radiate form to the colony as a whole. While this is intrinsically a matter of the biology of the fungus and not of the disease process, such as should carry most weight in denominating a new disease of unknown etiology, the precedent has been already set in the case of the radiate fungus and actinomycosis. In line with the best and most conservative thought, pending more accurate determination of the identity of the fungus of the capybara, judgment is suspended as to the name of the disease.

*Chromoblastomycosis.*—This is introduced here because like the capybara, it is indigenous to Brazil, and its essential fungus cell is

similar to the extent of being a hyphomycete. It is caused by *Acrotheca pedrosoi*<sup>8</sup> and *Phialophora verrucosa*.<sup>9</sup> Both in culture and tissue the organisms are brownish black, whence the name "chromoblastomycosis" applied to the disease which they cause. One North American case,

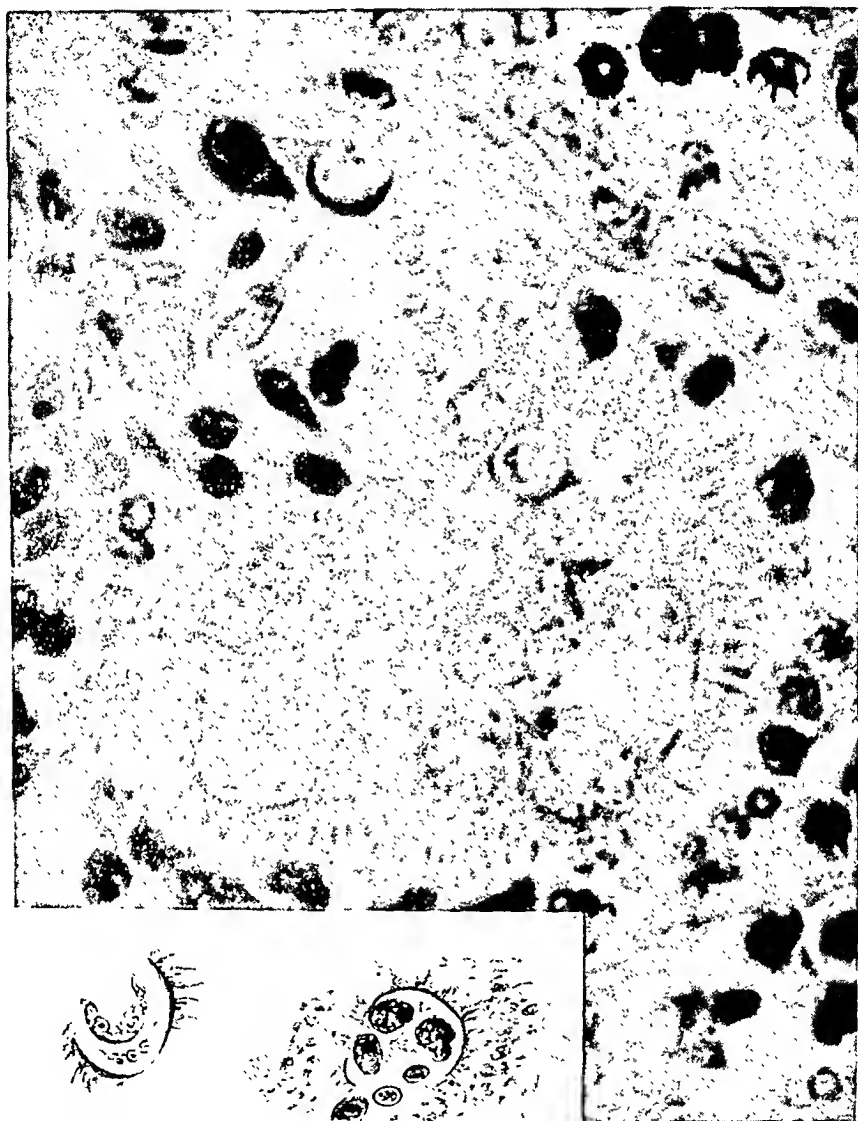


Fig. 6.—*Coccidioides immitis*. It shows radiate extensions from the shell of a ripe, ruptured cell containing numerous spores. The inserts are from Florence E. Ahlfeldt's paper (*J. Infect. Dis.* 44:277, 1929). Dr. Ahlfeldt supplied the photomicrograph.

due to *Phialophora verrucosa*, has been described. Chromoblastomycosis is effectively separable from pseudo-actinomycosis, partly because the

8. da Fonseca, O., and de Area Leao, A. F.: *Rev. de méd. y cir. da Brazil* 38:197, 1930.

9. Medlar, E. M.: *J. M. Research* 32:507, 1915.

former is known to occur only in the skin, but particularly because radiate fungus forms have never been described for it. It will be recalled that da Fonseca exchanged sections with me and emphatically declared that they are different, to which I agree.

*Aspergillosis.*—Aspergillosis of man as a primary, etiologically uncomplicated process is decidedly rare; as an accompaniment of old infarcts, bronchitis and particularly tuberculosis, it is commonly found if searched for. In progress, primary, uncomplicated aspergillosis of the lungs is excessively slow—up to fifty-two years in the case of a lesion affecting, say, half a pulmonary lobe.<sup>10</sup> I did not find granulomas described anywhere for human lungs such as were seen in the capybara; even in experimental animals, granulomas do not appear in the lung. In man, the pulmonary lesions appear essentially as bronchiectases or as edematous necrosis; in either case, intense bronchopneumonia surrounds such lesions.

As to radiate aspergillotic forms, they appear to have been seen in man but twice. Kohn<sup>11</sup> reported finding in histologic sections of lungs “grains” like actinomycosis, and stated that “long slender filaments (faden) extend out from its periphery.” He gave no additional information on which to base a comparison with the radiate form found in the capybara.

Boyce<sup>12</sup> similarly reported agglomerations in sections of the lung in which the nodule consisted almost entirely of mycelium. His illustrations depicted little if any hyaloid incrusting material; certainly, the appearances were not comparable with the radiate form in the capybara. The radiation effect in his “grains” was thus only indifferently expressed; for the most part, it was referable to the radiate arrangement of mycelium that is common to colonies of fungi in cultures. However, the terminal filaments exhibited swellings, which revives the subject of actinomycotic clubs. Unlike the latter, however, they were composed essentially of genuine, living fungus protoplasm and were not of incrustive nature.

Both as to type of tissue reaction and as to morphology of fungus, then, the aspergillosis of the capybara differed widely from that of man; indeed, were it not for the sufficiency of experimental infections in lower animals that so definitely incriminate *Aspergillus*, it would have been impossible to correlate the two hosts in the absence of cultures.

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10. Nicaud, P.: *Paris méd.* **1**:163, 1928.

11. Kohn, H.: *Deutsche med. Wchnschr.* **50**:1332, 1893.

12. Boyce, R.: *J. Path. & Bact.* **1**:163, 1893.

## RECONCILIATION OF ANIMAL PARASITIC AND CULTURAL FORMS

Commonly, the identification of hyphae in tissue carries the pathologist little farther than the diagnosis of mycosis; however, he is always curious as to whether similar or analogous forms are met in culture or in nature, and if so, whether they have value in identification of species. The morphologic ramifications of fungi are so manifold that the logical approach to the problem of determinative mycology in general is the other way around, i. e., through a knowledge of the habits of fungi and, after that, of the ways in which they become altered when transferred to tissue. This is seldom practical, however; mycology is almost a specialty in itself, and one that can be more reasonably dispensed with as a part of the equipment of the overloaded general pathologist. It still remains, however, that of the manifold mycobiologic phases, incrustment is a common and comparatively distinctive one ensuing when the fungus infests animal tissue; this at once commands the attention of the pathologist and justifies consideration of at least that phase.

## INCRUSTMENT IN GENERAL

*Taxonomic Distribution.*—The bacteriologist and protozoologist are already familiar with capsule and gloae formation (*Bacillus mucosus* group, the staphylococci of botryomycosis, *Myxosporidia*). In medical mycology it is also a well known phenomenon. Not to exhaust the list, it is expressed on *Saccharomyces hominis*<sup>13</sup> and *Torula histolytica*<sup>14</sup>—in the case of the latter, both in tissue and in culture. Minor, more or less denticulate or even radiate extensions are commonly noted on *Coccidioides immitis*<sup>15</sup> and its congener, *Zymonema braziliensis*,<sup>16</sup> in tissue. Echinulate, granular projections commonly occur from the exterior of fuseaux of microsporons and other dermatophytes in culture (Sabouraud<sup>17</sup>).

It has probably been seen in connection with an aspergillus, as a thick membrane around terminal swelling of hyphae, in a case of Kaposi's sarcoma (Dillard and Weidman<sup>18</sup>). Limited as we were to histologic sections, the question arose whether the formations were expressions of calcification, i. e., deposits from host tissue outside the fungus cell, or were products from its interior. It was a simple matter

13. Castellani, A., and Chalmers, A.: *Manual of Tropical Medicine*, ed. 3, New York, William Wood & Company, 1920, p. 1074.

14. Freeman, W., and Weidman, F. D.: *Arch. Neurol. & Psychiat.* **9**:589, 1923.

15. Ahlfeldt, F. E.: *J. Infect. Dis.* **44**:277, 1929; *Arch. Path.* **2**:206, 1926.

16. Almeida, F. P.: *Ann. de méd. de Sao Paulo* **4**:91, 1929.

17. Sabouraud, R.: *Les teignes*, Paris, Masson & Cie, 1910, p. 679.

18. Dillard, J. G., and Weidman, F. D.: *Arch. Dermat. & Syph.* **11**:203, 1925.

to refer such formations to terminal chlamydospores, but the degree of thickening was out of proportion to anything with which we were familiar. Turning now to the botanical literature, in following through the morphologic vagaries of *Aspergillus* one meets the Hülle cell<sup>19</sup> (an abortive fruit stalk) figured by Thom and Church,<sup>20</sup> and on following their reference to original work by Eidam,<sup>21a</sup> forms similar to those in the sarcoma were found figured for *Aspergillus* (*Sterigmatocystis*) *nidulans*. Eidam worked with materials that were free from parasitic environment, and in his opinion the membrane was developed from the protoplasm in the interior of the cell.<sup>21b</sup> According to this information, membranes are capable of developing around fungus cells entirely apart from parasitic environment.

*Nature and Source.*—One phase of incrustment is illustrated on conidia. In culture, the granular excrescences on conidia (*Scopulariopsis*, *Aspergillus*) and on the fuseaux of various dermatophytes<sup>17</sup> give incrustation effects. Thom and Church,<sup>22</sup> in their description of the genesis of the conidium, explain such granules or other masses on the basis that they correspond to substances formed in the wall of the conidium that "give coarseness or body to the roughenings or spinulosity." This observation, of course, connotes a source within the fungus cell.

From the medical angle, interest as to the nature and source of the incrusting material has centered mainly around a second form, namely, the radiate fungus of actinomycosis. Langeron, Cauchemex and Alleaux<sup>23</sup> analyzed the situation in 1925. They pointed out first that there was no uniformity in the distribution, taxonomically speaking, of such matter; it appeared around bacteria (the staphylococci of botrymycosis and *Actinobacillus lignieresii*) as well as around filamentous forms, such as *Actinomyces*. Even such high forms as *Monilia*<sup>6</sup> and *Sporotrichum*<sup>24</sup> could show clubs<sup>25</sup>; that is, there was nothing peculiar

19. Thom agrees with Eidam; he wrote me that he believed Hülle cells to be "morphologically undeveloped stalks" (of certain reproductive structures).

20. Thom, C., and Church, M.: *The Aspergilli*, Baltimore, Williams & Wilkins Company, 1926, p. 21.

21. Eidam, E.: (a) *Beitr. z. Biol. d. Pflanz.* **3**:377, 1883; (b) *ibid.*, p. 405.

22. Thom, C., and Church, M.: *The Penicillia*, Baltimore, Williams & Wilkins Company, 1930, p. 5.

23. Langeron, M.; Cauchemex, L., and Alleaux, V.: *Ann. de parasitol.* **3**:225, 1925.

24. Pinoy, E.: *Bull. Inst. Pasteur* **11**:929 and 977, 1913.

25. The original citation simply mentions *Sporotrichum* as a producer of clubs, without description or illustration. Subsequent authors, in repeating this citation, have given the species equal dignity with *Actinobacillus* as a club producer—an impression that is quite unjustified.



or specific (botanically speaking) in the formation of such matter. Incidentally, arrangement of this material in clubs or other radiate forms is likewise inconstant; with others, I have seen "grains" of Actinomyces, some of which exhibited clubs and others of which did not. In one instance, the radiate fungus exhibited clubs only at scattered points of the periphery. There are thus no rigid lines in respect to presence or absence of clubs. It still remains, however, that certain species of fungi feature clubs and rays more regularly than others; but one should always be ready and willing to recognize and accept exceptions to the rule.

The second interest of the authors just cited lay in the source and nature of the incrustment. Briefly, Pinoy's (cited by Langeron and his associates<sup>23</sup>) theory was that it was "a mixed formation which comes about through the digestive action exercised by the host upon the membrane of the parasite." On the other hand, Lignieres and Spitz<sup>26</sup> regarded it (clubs at least) as "young protoplasm, capable of budding and serving as pabulum for the filaments in the interior"; i. e., anything but expressions of degeneration or involution. The success that Bayne-Jones<sup>27</sup> met, confirmed by Langeron and his associates,<sup>23</sup> in inducing the formation of clubs in cultures appears to eliminate Pinoy's theory. It does not prove the additional contentions of Brumpt (quoted by Langeron and his associates<sup>23</sup>), particularly the one endowing the incrustment with vital properties. Bayne-Jones'<sup>27</sup> observation of filaments extending from clubs (in moist chamber) did not strengthen Brumpt's hypothesis; they sprang from filaments at the proximal end of the club rather than from the hyaloid substance of the club proper.

For Actinomyces and other slender organisms like the bacteria, I feel that the incentive for the development of the incrustations has not been proved. It is scarcely likely that *Bacterium actinomycetum-comitans* is the basis of the matter (having undergone lysis and coagulation), because it would have been detected long ago.

As seen in tissue, it is tempting to refer at once all of these encapsulation effects to the principles of the "Dauercyst" or resting cell so well known in protozoology; i. e., that the capsule is protective, a reaction against adversity such as may also be assumed for fungi in the comparatively anaerobic and torrid environment of mammalian tissue. However, it becomes necessary here to recall that capsule formation can appear under circumstances other than adversity. For instance, it

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26. Lignieres, J., and Spitz, J.: *Bull. Soc. centr. de méd. vét.* **20**:487 and 546, 1902; *Rev. Soc. méd. argent.* **11**:105, 1902.

27. Bayne-Jones, S.: *J. Bact.* **10**:569, 1925.

occurs preparatory to reproduction, as in (1) the formation of cysts among the protozoa and in (2) the formation of asci among fungi.

As to the particular aspergillotic radiate fungi described by Macaigne and Nicaud,<sup>5</sup> and the form isolated from the capybara, there is an explanation that I would emphasize in an entirely different direction; namely, that the incrustation represents suppressed formations of the order of the Hülle cells (p. 739). One is struck at the bulk of the incrusting matter enclosing the delicate filament that presumably produced it;<sup>28</sup> it is tempting, indeed, to assume that products of the micro-organism have diffused outward and coagulated or otherwise hyalinized surrounding fluids. This theory deserves serious consideration with the others.

*Conclusion in Re Incrustment.*—It appears, thus, that incrustment of fungus cells in mammalian tissue can be brought about in more than one way:

1. Incrustment is a measure of resistance against adverse environment (this is illustrated in *Torula histolytica*); or, if the situation is viewed in a less vitalistic light it is due to the action of diffusion products (coagulating?) of the cell that provokes the deposit of surrounding fluids of other substances on the cell membrane.

2. It is an accompaniment of reproductive processes. The reproductive structure that it accompanies may be (a) developed fully, as with *Coccidioides immitis*, or (b) suppressed. Of this, final proof is lacking, but the Hülle cells of *Aspergillus* resemble forms seen in human tissue and may be tentatively classified, together with radiate aspergillotic forms under this heading.

It is appreciated that the first and second possibilities are interrelated to a certain extent, because the onset of adverse conditions is often the factor that initiates reproductive formation of cysts. However, the possibility of the absolute independence of the environmental factor from the reproductive factor, and the acquirement of marked and additional morphologic changes when incrustment of the latter type succeeds on that of the former type justify their separation.

*Radiate (per Se) Effects.*—The radiate or quasiradiate effect as it appears in these different forms of incrustment is contingent on at least three different circumstances. 1. In the torula (*histolytica*) cited, it was due to radial condensation within a common hyaloid stratum; it was not radiate in point of (configuration of) mass, but only in point of coloration (and perhaps also of chemistry). 2. With *Coccidioides immitis* and the fungus of the capybara, the capsule occurs not as a shell

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28. Bayne-Jones' illustration clearly depicts the filament extending as the axis of the club, in culture.

of uniform thickness, but as a covering of radiating denticulations or even of elongated rays. 3. With *Actinomyces* it would be expeditious to explain the radiation simply as incidental to the radiate arrangement of mycelial filaments common to colonies of fungi in general, the hyaloid incrustment being continued like a coat of ice over the surface of the colony and its projecting terminal hyphae. Actually, such hyphae can seldom be demonstrated in the axis of a club, although Magrou<sup>6</sup> figured them for *Botryomyces*. However, in the light of what the fungus in the capybara showed as to the capacity of a solitary hypha to develop multiple radiations, it is now in order to strengthen the concept that the radiations of *Actinomyces* need never have contained central filaments but consisted of solid incrustment substance which was independently radiate from the first.

*Calcification.*—This may complicate true mycologic incrustation; not only does this modify staining reactions, but the thickening may be exaggerated or distorted. This occurred in the case of Kaposi's sarcoma cited. Certain blastomycetoid bodies occurring in a sarcoma-like lesion and in lupus vulgaris exhibited such concentric effects that they resembled psammoma bodies (Weidman and Douglas<sup>29</sup>). The much debated Gamna-Gandy bodies of African splenomegaly<sup>30</sup> are also in point. In all of these cases there is a question as to the genuineness of their fungous nature; elastic fibers are notoriously liable to calcification, and thus mimic calcified hyphae. The color plates of Boyce are most welcome in this dilemma, because they demonstrate filaments (in experimentally inoculated animals) with thickened, basic-stained walls within giant cells analogous to or identical with, at least morphologically, some of the "unknowns" cited. Boyce<sup>12</sup> enables one to diagnose hyphae within giant cells more confidently (see his fig. 8).

*Classification of Incrustment.*—The tabulation appended is on a morphologic basis; it is designed to show the range as to (1) quantity, (2) configuration (diffuse or radiate) and (3) species of fungus involved.

A. Radiation effect absent

1. Gloea formation (*Botryomyces*<sup>6</sup>)
2. Capsule formation (*B. mucosus-capsulatus*, *Torula histolytica*<sup>14</sup>),  
*Saccharomyces hominis*<sup>13</sup>

29. Weidman, F. D., and Douglas, H. R.: *Arch. Dermat. & Syph.* **3**:743, 1921.

30. da Fonseca, O., and de Area Leao, A. E.: *Mem. do Inst. Oswaldo Cruz*, Aug. 31, 1928, supp. 1, p. 10. Symmers, D.; Gettler, A. O., and Johnson, W. M.: *Surg., Gynec. & Obst.* **28**:58, 1919. Nanta, E., and Pinoy, P.: *Presse méd.* **36**: 579, 1928.

## 3. Granular projections on cell membranes

- (a) Conidia and fuseaux of sundry dermatophytes (Achorion, Microsporon<sup>17</sup>)
- (b) Madurella
- (c) Fürbringer's "involutive conidiophores"<sup>31</sup>

## B. Radiation effect present

- 1. A staining reaction: radiations not due to radial arrangement of incrusting substance, but to radially distributed variations in staining reaction (T. histolytica)
- 2. Incrusting substance arranged radially<sup>32</sup>
  - (a) No clubs. The matrical substance is more or less finely granular; its granules are arranged radially or in striae (some members of Actinomyces)
  - (b) Clubs at periphery extend radially,<sup>33</sup> either completely around periphery (Actinomycosis, Actinobacillus,<sup>26</sup> Aspergillus) or incompletely around periphery (Oidium,<sup>6</sup> Actinomycosis)
  - (c) Incrusting substance calcareous; radiation effect due to hyphae (Tonsillar and lachrymal calculi Kapsenberg<sup>34</sup>)

## SUMMARY AND CONCLUSIONS

In the lungs of a capybara a radiate form of fungus developed which was definitely different from that of actinomycosis both in certain features of its general morphology and in the identity of the fungus at its basis. While the fungus could not be classified with finality (on account of absence of cultures), it is suspected that it belongs to *Aspergillus*.

The infection was possibly acquired during an epizootic of aspergillosis among nearby penguins.

Since the radiate effect was found to be due to hyaloid material deposited on the exterior of the mycelium, the opportunity was taken to compare and analyze such deposits on fungi in general, both in culture and in tissue. As they occur in culture, it is concluded that

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31. Fürbringer: Virchows Arch. f. path. Anat. **66**:330, 1876.

32. The reactive host cells immediately adjacent to the radiate fungus are often arranged radially-strially, adding to the radiate effect. In absence of incrustment or matrical substance, radiation effects may still be met: mycelial hyphae arranged radially in tissue as they are in culture (Davis' actinomyces-like "grains" of tonsils [J. Infect. Dis. **14**:144, 1914], Boyce's<sup>12</sup> aspergillosis of human lung, *Mycobacterium tuberculosis*, *M. diphtheriae*).

33. Clubs may be feathery in form or show lateral spinules. In no case are all of the clubs spinulose; commonly only a few are thus affected (*Oidium*,<sup>6</sup> *Botryomyces*,<sup>6</sup> the capybara's *Aspergillus*).

34. Kapsenberg, G.: Tijdschr. v. vergelijik. geneesk. **9**:249, 1923.

those occurring in tissue, as in actinomycosis and aspergillosis, are not deposited from the surrounding tissue. The process is not analogous to the incrustments occurring in calcification.

Incrustment of fungus organisms in tissue involves a wide range of species—bacteria, streptothrixes, yeastlike forms and aspergilli. In addition, a radiate arrangement of such more or less matrical material can likewise occur in a variety of species (*Actinomyces*, *Actinobacillus*, *Aspergillus*). Fungus in a radiating form is not peculiar to or pathognomonic of actinomycosis; it serves only to focus attention on a particular group of micro-organisms, numerically small, but taxonomically widely distributed.

While the radiations are generally club-shaped, they may have more or less rectilinear sides, be spicular or otherwise depart from the conventions expressed in the radiate fungus *Actinomyces bovis*.

A table or key is submitted in which an attempt is made to classify the different expressions of incrustment according to morphology and species concerned.<sup>35</sup>

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35. Histologic sections of the lung of the capybara are deposited at the Army Medical School, Washington, D. C. (no. 36460), at the Laboratory of Comparative Pathology, Philadelphia Zoological Garden (no. 9998), and at the Laboratory of Dermatological Research, University of Pennsylvania (no. 2226).

# TISSUE CHANGES FOLLOWING CONTINUOUS INTRA- VENOUS INJECTION OF EPINEPHRINE HYDROCHLORIDE INTO DOGS

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It is the purpose in this paper to describe certain tissue changes that occurred in dogs following the intravenous administration of epinephrine hydrochloride at constant low rates over relatively long periods of time. Chemical data obtained in the study of several of the dogs have been presented in a previous paper.<sup>1</sup>

Consistent histologic changes were demonstrated in the liver, kidneys, hypophysis and adrenal glands. None were found in the spleen, striated muscle, smooth muscle, lungs, aorta or pancreas. The results obtained from staining sections of striated muscle for glycogen were inconclusive and could not be interpreted. Because of the lack of adequate controls of known age, the changes in the thymus could not be regarded as due to the injection of epinephrine hydrochloride and are therefore not included.

As far as can be determined, any tissue changes previously reported have occurred as the result of repeated injections of large amounts of epinephrine hydrochloride. In this connection, the work of Drummond<sup>2</sup> and that of Herter and Richards<sup>3</sup> may be mentioned. Acute changes such as congestion, hemorrhage and edema were prominent in their experiments. Drummond, giving from 10 to 15 cc. of 1:1,000 solution at a time, found no changes in the pancreas, spleen or adrenal glands. In the kidneys, he described widespread cloudy swelling in the lining epithelium of the convoluted tubules. In the liver, he noted central lobular necrosis and fatty changes in the cells in the middle zone of the lobule. The lungs were edematous, and an occasional polymorphonuclear leukocyte was found in the fluid. Herter and Richards described focal necroses in the pancreas and destructive changes in the islets following intraperitoneal injection of a single fatal dose of epinephrine hydrochloride.

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Submitted for publication, Dec. 7, 1931.

From the Department of Medicine, Rush Medical College of the University of Chicago.

1. Samson, P. C., and Jacobs, H. R. D.: *Am. J. Physiol.* **99**:433, 1932.
2. Drummond, W. B.: *J. Physiol.* **31**:81, 1904.
3. Herter, C. A., and Richards, A. N.: *M. News* **80**:201, 1902.

## METHODS

*Injections.*—The apparatus used for the injections has been described by Jacobs.<sup>4</sup> Under local anesthesia, a cannula was inserted into the jugular vein of the dog, and epinephrine diluted with physiologic solution of sodium chloride was injected continuously at rates considered to be well within the maximum physiologic rate of secretion of the adrenal glands (Cannon and Rapport<sup>5</sup> and Kodama<sup>6</sup>). The rates of injection used in the present investigation were from 0.001 to 0.002 mg. per kilogram per minute (0.06 to 0.12 mg. per kilogram per hour), based on the original weight of the dog.

Medium-sized dogs of both sexes were used. They were unrestricted except by the collar and chain of the apparatus. They were given no food during the injection, but were allowed water as desired. At the end of the experiment, the dogs were killed, and a postmortem examination was made immediately. Tissues were fixed at this time for subsequent microscopic examination.

A commercial 1:1,000 dilution of epinephrine hydrochloride was used.

*Fixation and Staining.*<sup>7</sup>—For general survey, tissues were fixed in stock Zenker's solution or Zenker's solution plus solution of formaldehyde and paraffin blocks prepared according to the usual procedure. Sections were cut from 6 to 8 microns thick, made adherent by the albumin fixative method, stained with Delafield's iron-hematoxylin and counterstained with aqueous eosin.

For more detailed observation, the following stains were used: (1) Altmann's aniline-water-acid-fuchsin and aqueous methyl green on sections of pancreas, hypophysis and adrenal gland; (2) Mallory's reticulum stain on sections of hypophysis and aorta, and (3) toluidine blue, alone and with aqueous safranin, on sections of adrenal gland.

The fats were preserved in certain tissues by fixation with a diluted solution of formaldehyde, U. S. P. (1:10). Sections were cut on the freezing microtome and stained with an acetone solution of sudan III, followed by hematoxylin.

For the detection of glycogen, tissues were fixed in absolute alcohol. Sections were cut from paraffin blocks and stained with Best's carmine mixture, with the use of hematoxylin and a nuclear stain.

In addition to the fixative agents mentioned, pancreatic tissue was fixed in trinitrophenol-formaldehyde solution (so-called picro-formol<sup>7a</sup> and in acetic-osmic-bichromate solution (as used by Bensley<sup>8</sup>). Bowie's stain<sup>9</sup> was employed on the sections. The sections were removed from the staining solution at the end of

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4. Jacobs, H. R. D.: J. Lab. & Clin. Med. **16**:901, 1931.

5. Cannon, W. B., and Rapport, D.: Am. J. Physiol. **58**:308, 1921.

6. Kodama, S.: Tohoku J. Exper. Med. **4**:166, 1923-1924.

7. For a complete description of the fixative agents and stains used, see the following: Warthin, A. S.: Practical Pathology, ed. 2, Ann Arbor, Mich., George Wahr, 1921. Mallory, F. R., and Wright, J. H.: Pathological Technique, ed. 6, Philadelphia, W. B. Saunders Company, 1913. Lee, B.: The Microtome's Vade-Mecum, ed. 9, Philadelphia, P. Blakiston's Son & Co., 1928.

7a. The solution to which this term was applied by P. Bouin is made up as follows: trinitrophenol saturated aqueous solution, 75 parts; solution of formaldehyde, 25 parts; acetic acid, 5 parts.

8. Bensley, R. R.: Am. J. Anat. **12**:297, 1911.

9. Bowie, D. J.: Anat. Rec. **29**:57, 1924-1925.

twenty-four hours, blotted with absolute alcohol, and differentiated in pure oil of cloves. The differentiation was controlled under the microscope.

In staining for elastic fibers, Weigert's resorcin-fuchsin was used, following lithium carmine.

#### PROTOCOLS

Continuous injections of epinephrine hydrochloride were made in one male and five nonpregnant female dogs. The length of the injections varied from 120 to 289 hours. The amount injected varied from 59 to 246 mg. The average loss in weight of the six dogs was 159 Gm. per day. Control tissues were taken from: (1) ten normal dogs (six females and four males); (2) two dogs into which physiologic solution of sodium chloride had been injected continuously for fourteen and fifteen days, at rates of from 20 to 50 cc. per hour. The average loss in weight of the latter two dogs was 191 Gm. per day. In three of the experimental dogs, a terminal pulmonary infection developed. They are included for the reason that the tissue changes in the other organs were similar to those in the rest of the dogs given injections.

#### RESULTS

*Liver.*—The changes in the liver were all of the same type, but were not in proportion to the amounts of epinephrine hydrochloride injected. Grossly, the livers were smaller and more friable than normal and showed a distinct yellow mottling on cut surfaces.

A comparison of sections from the different livers stained with hematoxylin and eosin showed that in some the change was limited to a slight degree of vacuolization in the cells immediately surrounding the central vein of the lobule. In those livers showing the most change, nearly all the cells were vacuolated. The cytoplasm of the more central cells of the lobule was entirely replaced, only cell outlines being visible. All nuclei stained well (fig. 1).

In sections of liver stained with sudan III following fixation in formaldehyde, the vacuoles and clear spaces were filled with fat. The degeneration was a fatty change and not necrosis. No glycogen was demonstrable by staining in any of the animals. Since the livers of the control dogs, into which physiologic solution of sodium chloride had been injected continuously, contained enough glycogen to give a faintly positive staining reaction, even after two weeks without food, it is probable that if the livers of the experimental animals contained glycogen, it was present in much less than normal amount.

*Kidneys.*—On gross examination, the kidneys were generally paler than normal. The capsule stripped easily. The chief gross changes were in the cortex. In three of the animals there was a yellowish band in the middle zone of the cortex, and in one, the whole cortex was distinctly yellow in contrast with the normal pink of the medulla.

The microscopic changes varied in amount, but were of the same nature in all. They were of the type found in nearly every "normal" dog, but were much greater in degree. Stained with hematoxylin and eosin, the different kidneys showed varying degrees of vacuolization in the cells lining the limbs of Henle's loop. In many of the cells, the cytoplasmic area was entirely clear. The nuclei stained well. Congestion was generalized in the capillaries of the cortex. The convoluted tubules and glomeruli appeared normal (fig. 2). Sections stained with sudan III demonstrated a fatty change in the cells lining the limbs of Henle's loop. The change was marked throughout the cortex, but ended abruptly at the junction of the cortex and medulla. The orange streaks were visible grossly on the slide.



A small amount of fat was occasionally found in the cells of the collecting tubules, a condition not seen in any of the sections from normal animals. No other changes were noted in the medulla. Glycogen degeneration was not demonstrated.

*Hypophysis Cerebri.*—There seemed to be no variation in the size either of the anterior lobe or of the hypophysis as a whole when glands from the experimental animals were compared with normal glands. However, no exact weights were obtained. In sections that were stained with hematoxylin and eosin, the anterior

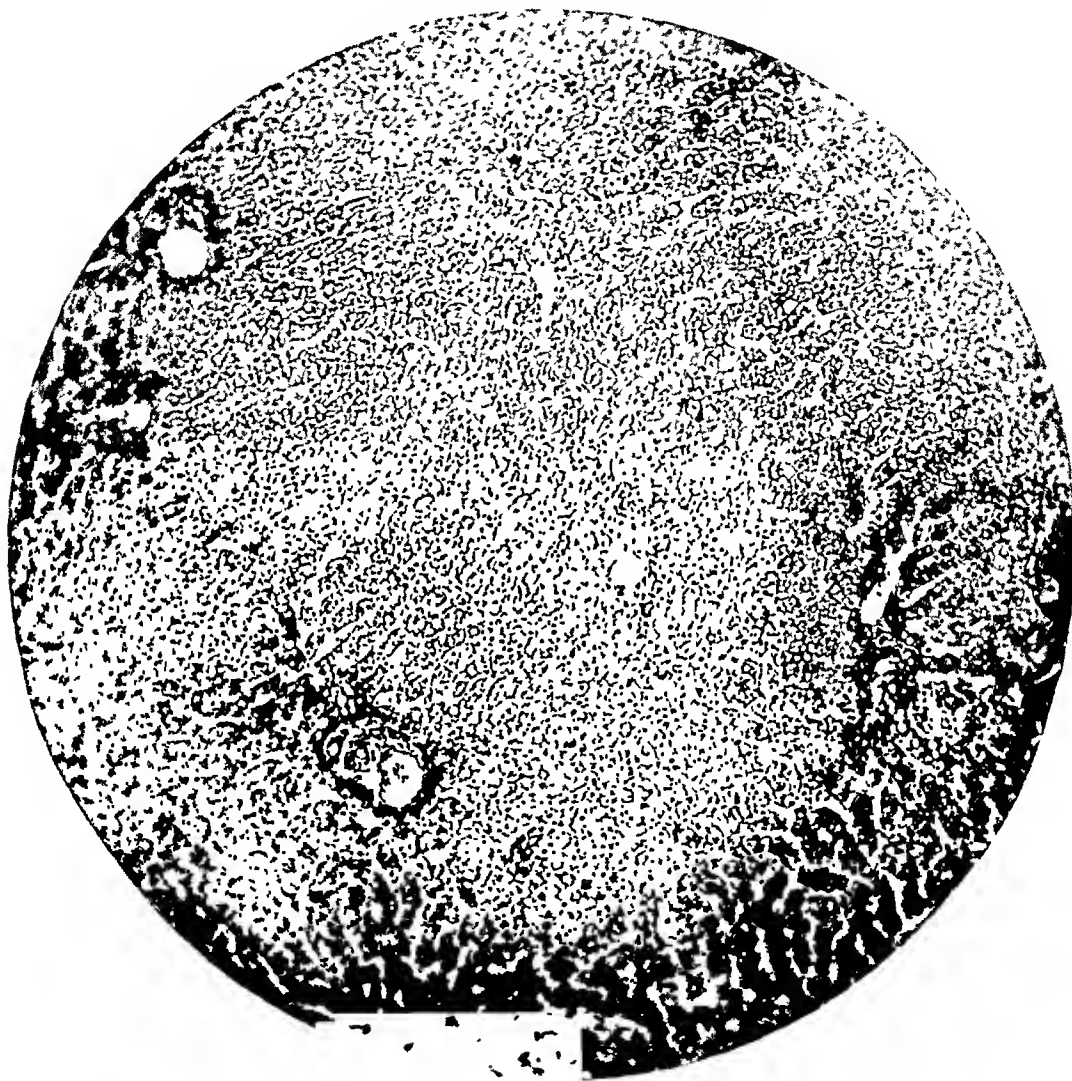


Fig. 1.—A section of liver stained in hematoxylin and eosin and magnified 80 times, from a dog that received 59 mg. of epinephrine hydrochloride in 184 hours. It shows extensive vacuolization of the central lobular cells and partial vacuolization of the more peripheral cells.

lobe tended to take a heavy eosin stain and showed in general more nuclei per high power field than were found in the controls. The blood spaces did not appear to be consistently more dilated or more collapsed than normal.

Examination of sections stained with 1 per cent aniline-acid-fuchsin and 0.5 per cent aqueous methyl green showed that there were consistent changes in the cellular elements of the pars anterior in all the experimental animals.

The chromophobe cells showed a considerable loss of secretory substance, which resulted in cytoplasmic shrinkage. This appeared to be the mechanism chiefly responsible for the increased number of nuclei seen in a single field.

The alpha cells were more intensely acidophilic and were present in greater numbers. They did not seem to be increased in size. No mitotic figures were observed. The alpha cells varied in their affinity for fuchsin. A few stained faintly, but the greater proportion were hyperacidophilic. The deeply staining

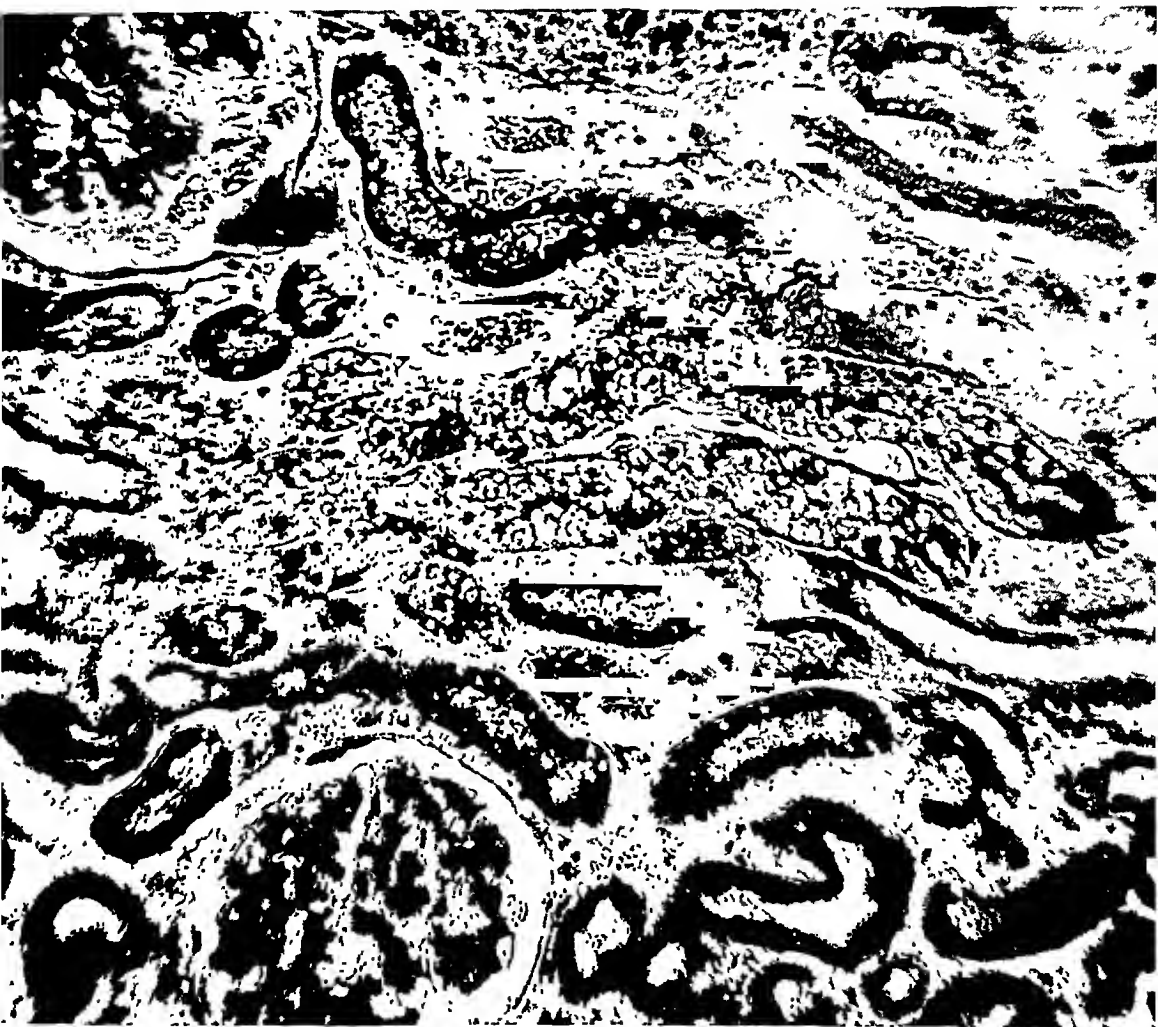


Fig. 2.—A section of kidney stained with hematoxylin and eosin and magnified 290 times, from a dog that received 69 mg. of epinephrine hydrochloride in 164 hours. It shows vacuolization of the cells lining the limbs of Henle's loop.

alpha cells were numerous in all parts of the anterior lobe and were found in what appeared to be definite layers surrounding many of the blood spaces (fig. 3). In the experimental animals, this condition was generalized throughout the anterior lobe and was conspicuous. In the normal animals, the alpha cells were found for the most part in the region bordering the pars intermedia, and the tendency to layer formation around the blood spaces was occasionally noted.

Kojima<sup>10</sup> (quoted by Cowdry<sup>11</sup> and Maximow<sup>12</sup>) gave epinephrine to animals in food. He reported an enlargement of the anterior lobe and an increase in both the size and the number of the alpha cells. On the contrary, Igura,<sup>13</sup> examining rats after the injection of epinephrine hydrochloride described a decrease in the size and number of the alpha cells and an enlargement of the chief cells.

In sections from the experimental animals stained with Mallory's reticulum stain, the basophilic cells appeared to be smaller and diminished in number; in fact, they were often difficult to find.

*Adrenal Glands.*—Grossly, the adrenal glands appeared normal in size and consistency. The amount of congestion varied, but was within normal limits. There were no gross hemorrhages. The hematoxylin and eosin stains were normal. A few red blood cells were scattered along the cell columns of the cortex. Sections stained with sudan III did not show any alteration in the quantity, type or distribution of the lipid substance in the cortex, when compared with the normal. No glycogen degeneration was demonstrated.

Borberg<sup>14</sup> and Ogata and Ogata<sup>15</sup> showed that when sections of adrenal tissue are stained with toluidine blue, the depth of green that develops in the medulla is roughly proportional to the amount of epinephrine or preepinephrine substance present. Such tissues must be fixed in some acid-free chromate solution, since acid seems to interfere. The latter authors believed the reaction to be a simple reduction of the epinephrine on the chromates.

Sections of adrenal glands were fixed in Zenker's solution plus solution of formaldehyde and stained with 0.5 per cent aqueous toluidine blue. In all cases, the depth of green that developed was less than that found in the normal controls. In the adrenal glands from two of the experimental animals, only the faintest traces of green could be found in the medulla. Such findings agree with those of Tournade and Chabrol,<sup>16</sup> and Molinelli,<sup>17</sup> who concluded from chemical data that injection of epinephrine hydrochloride results in diminution of the endogenous secretion.

In staining with toluidine blue it was found better not to counterstain with safranin and dehydrate in alcohol, as some authors recommend. As pointed out by Ogata, alcohol causes toluidine blue to fade. After the excess toluidine blue had been washed out, the slides were blotted with successive applications of xylene until they became translucent; thus the necessity for using alcohol in dehydration was avoided.

A consistent change in staining reaction that was rarely found in the normal control was demonstrated in certain cells of the adrenal cortex of the experimental animal when sections were stained with 1 per cent aniline-acid-fuchsin and 0.5 per cent aqueous methyl green. These cells were similar to the surrounding cells

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10. Kojima, M.: *Quart. J. Exper. Physiol.* **11**:319, 1917.

11. Cowdry, E. V., in Barker, L. F., and others: *Endocrinology and Metabolism*, Philadelphia, W. B. Saunders Company, 1922, vol. 1, p. 705.

12. Maximow, A. A.: *Text-Book of Histology*, finished and edited by William Bloom, Philadelphia, W. B. Saunders Company, 1930, p. 691.

13. Igura, S.: *Abstr., Ber. ges. Physiol. u. exper. Pharmacol.* **44**:104, 1928; *Chem. Abstr.* **22**:2987, 1928.

14. Borberg, N. C.: *Skandinav. Arch. f. Physiol.* **28**:91, 1912.

15. Ogata, T., and Ogata, A.: *J. Exper. Med.* **25**:807, 1917.

16. Tournade, A., and Chabrol, M.: *Compt. rend. Soc. de biol.* **94**:535, 1926.

17. Molinelli, E. A.: *Compt. rend. Soc. de biol.* **95**:1084, 1926.

in size and shape and were scattered irregularly throughout the cortex. Mitosis was not observed. The cytoplasm was somewhat coarsely granular and intensely acidophilic, staining red in aniline-acid-fuchsin and blue in toluidine blue. The nuclei were smaller and somewhat more compact than those of the surrounding cortical cells and gave a metachromatic staining reaction. In aniline-acid-fuchsin and methyl green, they stained red or purple, and in toluidine blue and safranin, blue or red, depending on which stain was employed most intensely.

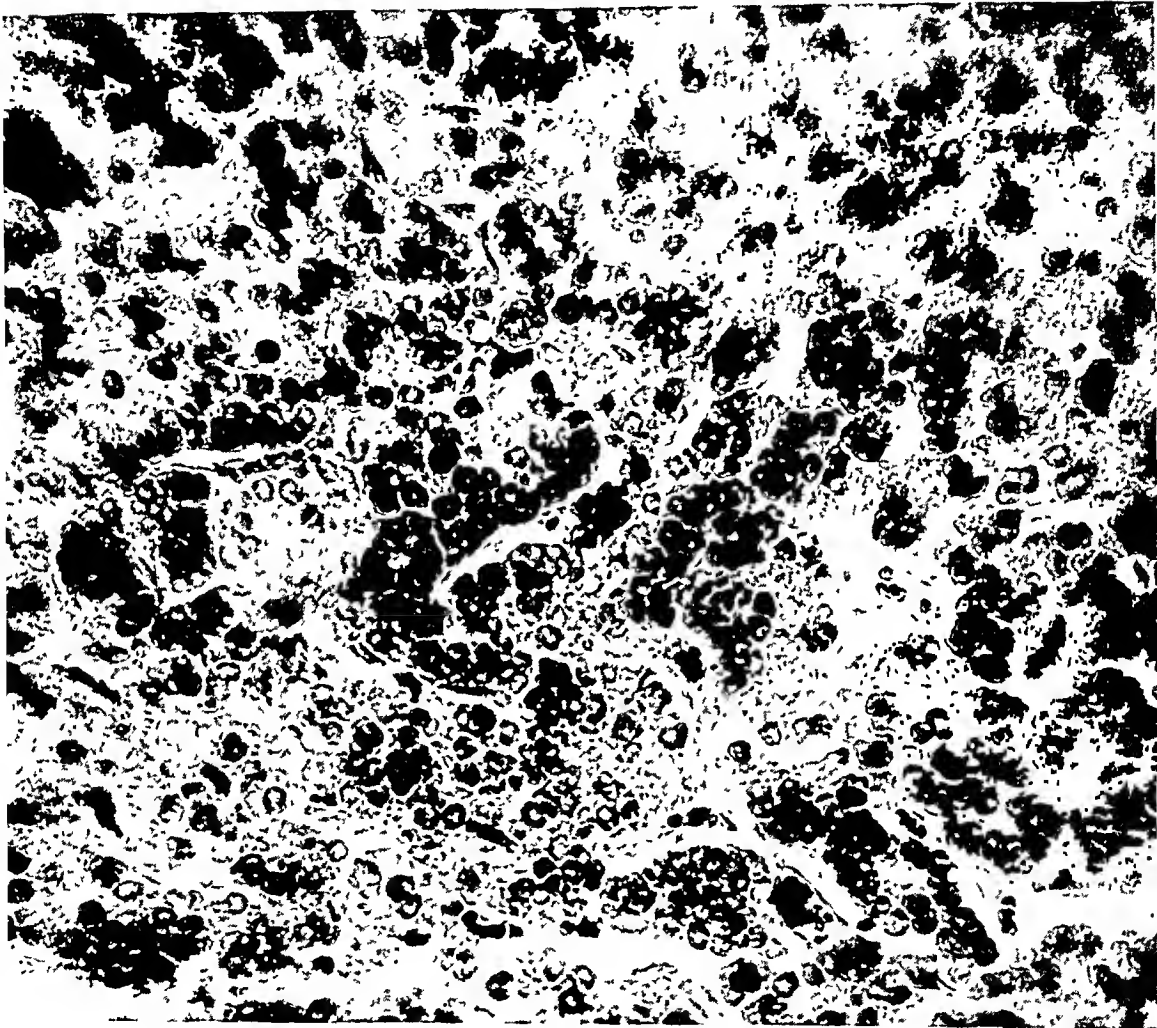


Fig. 3.—A section of hypophysis stained with aniline-acid-fuchsin and methyl green and magnified 320 times, from a dog that received 112 mg. of epinephrine hydrochloride in 272 hours. It shows by the dark cells the increased number of hyperstaining alpha cells. The distribution of alpha cells in layers around the blood spaces is particularly conspicuous.

*Cardiovascular System.*—In five dogs, the heart was found in systole. In the other, it was in diastole. In three of the animals, there were small punctate endocardial hemorrhages in the wall of the left ventricle, chiefly around the base of the papillary muscle. In three, the wall of the aorta appeared yellower than normal. In no case was there evidence in any vessel of intimal plaques or calcification.

Hematoxylin and eosin stains of the myocardium were normal. Weigert's resorcin-fuchsin stain showed that the internal elastic lamina of the coronary vessels was uninterrupted. No pathologic changes were found in the smaller arteries and veins. Since Josué<sup>18</sup> in 1903 first described aortic sclerosis following repeated injections of epinephrine hydrochloride, many authors have reported experimental atheromatous lesions produced by this method. The literature on this subject has been reviewed by MacCallum.<sup>19</sup> Lesions have been produced successfully in rabbits only. Pearce and Stanton<sup>20</sup> attempted to reproduce similar changes in the aorta of a dog by the injection of epinephrine hydrochloride. They gave about 9 mg. over a period of a month.

Although in the aortas from the experimental animals occasional areas were found where there were slight fragmentation of elastic fibers and some increase in connective tissue (Mallory's reticulum stain) in the outer third of the media, similar areas were also found in normal controls. The connective tissue was not hyalinized, and there was no increase or decrease in cellular elements in the questionable areas. No calcium or fatty deposits were noted. It was concluded that no definite changes occurred in the aorta that could be attributed to the action of the injected epinephrine hydrochloride.

*Pancreas.*—Because of reports in the literature by Miyairi,<sup>21</sup> who described islet hypertrophy and hyperplasia and a decrease in granule content of the islet beta cells following injection of epinephrine hydrochloride, and by Igura,<sup>13</sup> who described atrophy of the pancreas cells as the result of administration of epinephrine, the pancreas was studied with especial care. This phase of the work was carried on under the supervision of Prof. R. R. Bensley of the University of Chicago, who also reviewed the sections of the hypophysis and aorta. In several of the dogs, serial sections from 3 to 5 microns thick were cut from different blocks of pancreatic tissue fixed in the fluids outlined under "Methods." These were stained both with Bowie's solution and with aniline-acid-fuchsin and methyl green.

No histologic evidence was found that would indicate either islet degeneration or the formation of new islets from acinar tissue. Islet hypertrophy or hyperplasia was not demonstrated. The granule content of both the alpha and the beta islet cells appeared to be within normal limits in all the experimental animals when sections were compared with normal controls. The zymogen granule content of the acinar cells also appeared to be normal.

#### COMMENT

It is evident that the continuous intravenous administration of epinephrine hydrochloride over relatively long periods of time, even when the drug is injected at rates considered well within the maximum physiologic rate of secretion of the adrenal glands, has a deleterious effect on the animals used. There appears to be an individual susceptibility to epinephrine, since, in spite of the fact that the rate of injection was about the same for each animal, the degree of tissue change was not necessarily in proportion to the amount of epinephrine hydrochloride administered.

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18. Josué, O.: *Presse méd.* **11**:798, 1903.

19. MacCallum, W. G.: *Physiol. Rev.* **2**:70, 1922.

20. Pearce, R. M., and Stanton, E.: *J. Exper. Med.* **8**:74, 1906.

21. Miyairi, S.: *Proc. Imp. Acad.* **3**:702, 1927.

In watching the behavior of the animals given a continuous injection of epinephrine hydrochloride, and of those given an injection of physiologic solution of sodium chloride only, it was noted that in the former group the dogs became quiet rather early in the experiment and apparently became weaker as the experiment progressed, the weakness being most marked in those animals that afterward showed the greatest tissue change. The control animals, on the other hand, showed surprisingly little weakness and no changes in behavior even after continuous injection and starvation for a period of two weeks.

The action of epinephrine seems in part to be a toxic or a degenerative one and, as might be expected, the liver and kidneys show morphologically the greatest damage.

The reason for the cell changes in the anterior lobe of the hypophysis is not clear, since the exact function of the different cells is not known. There was an evident increase in the number of acidophilic cells as seen in sections from the experimental animals. A relative increase resulted from the loss of secretory substance of the chromophobe cells, which in itself appeared to follow the injection of epinephrine hydrochloride. The belief that there may have been an actual, as well as a relative, increase in the number of alpha cells, particularly of the hyperstaining type, is based on the following observations: It was found that, in addition to the large number of hyperacidophilic cells seen in each microscopic field, there were single cells and groups of cells scattered irregularly throughout the sections in which the cytoplasm was very faintly acidophilic. All gradations of intensity in staining were found. Because of morphologic similarities, these cells were all believed to be alpha cells in various stages of development. Since no mitotic figures were observed, it seems plausible that there was a transition to acidophils from cells already present in the anterior lobe, perhaps similar to the transition described by Collin,<sup>22</sup> the result of secretory activity.

Whether the immediate antecedents of the faintly staining acidophilic cells were undifferentiated cells, chromophobes or possibly basophils (since these were decreased markedly in the experimental animals), could not be decided from the evidence at hand. In addition, the conspicuous distribution of the hyperstaining alpha cells in more or less definite layers surrounding the blood spaces strengthened the view that many of these cells had but recently acquired the acidophilic characteristics. Such a distribution might also be taken as evidence that some substance in the blood stream, presumably epinephrine, was responsible for the increase in the number of acidophilic cells. It is impossible to say whether the cell changes in the anterior lobe were the result of stimulation or of toxic action of the injected epinephrine hydrochloride.

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22. Collin, R.: *Compt. rend. Soc. de biol.* **102**:853, 1929.

In view of the definite changes that have often been reported as taking place in the aorta of the rabbit following the administration of epinephrine hydrochloride it is surprising that no changes were found in the aorta of the dog. It is known that sclerosis and calcification of the aorta occur spontaneously in the rabbit, and that this condition is practically never found in the dog.

While one can discuss, from a theoretical point of view, the possible reasons for the lack of change found in the aorta of the dog following the administration of epinephrine hydrochloride, further experimentation must be done before definite conclusions can be drawn.

The aorta of the dog may have an intrinsic immunity to the action of epinephrine. It may be that the liver of the dog is more efficient in destroying epinephrine than that of the rabbit and is more injured thereby.

Kajimura<sup>23</sup> showed that the injection of epinephrine hydrochloride into rabbits causes hypercholesteremia, followed by fatty deposits and sclerotic changes in the aorta. It is not known whether the injection of epinephrine hydrochloride causes hypercholesteremia in the dog.

It should be determined whether or not epinephrine produces effects on the vasa vasorum in the rabbit and dog that are similar. Hartman and his co-workers<sup>24</sup> obtained a vasodilatation in several species of mammals following the injection of moderate amounts of epinephrine hydrochloride, but obtained only vasoconstriction in rabbits. They concluded that rabbits did not possess an epinephrine vasodilatation mechanism. Somewhat later, Ravault and Bouysset,<sup>25</sup> using rabbits, injected trypan blue both alone and immediately preceding the injection of epinephrine hydrochloride. In the latter case, the coloration of the outer zone of the media was much decreased. They concluded that there was constriction of the vasa vasorum following the injection of epinephrine hydrochloride, with consequent interference with the nutrition of the aortic wall. They produced sclerotic changes in the same region by numerous injections of epinephrine hydrochloride, which they believed were based on nutritional disturbances. To my knowledge, similar experiments have not been performed in the dog.

Whatever the reason, it is probable that the marked arterial changes that have been reported to follow the injection of epinephrine hydrochloride in the rabbit cannot be duplicated in the dog.

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23. Kajimura, S.: *Okayama-Igakkai-Zasshi* **41**:1854, 1929.

24. Hartman, F. A.; Kilborn, L. G., and Lang, R. S.: *Endocrinology* **2**:122, 1918.

25. Ravault, P. P., and Bouysset, C.: *Compt. rend. Soc. de biol.* **99**:828 and 829, 1928.

It is possible that the cell changes described in the adrenal glands may be the result of a toxic action of the injected epinephrine hydrochloride on the adrenal cortex.

#### SUMMARY

Epinephrine hydrochloride was administered intravenously to dogs at constant rates over relatively long periods of time (up to thirteen days). The amounts injected varied from 59 to 246 mg.

Consistent tissue changes were found in the liver, kidneys, hypophysis and adrenal glands. The pancreas, aorta, spleen, smooth and striated muscle and lungs were found unchanged. The liver showed an intracellular fatty change. In the cases in which the liver was the least affected, small droplets of fat were deposited in the cells surrounding the central vein of the lobule. With more advanced change, the peripheral cells of the lobules contained fat droplets, and the cytoplasm of the central cells was almost entirely replaced by fat. The kidneys showed a fatty change in the cells of Henle's loop and rarely in the cells of the collecting tubules. In the anterior lobe of the hypophysis there was loss of the secretory substance of the chromophobe cells. The alpha cells showed in general a hyperacidophilic staining reaction, appeared to be increased in number and showed a conspicuous distribution in layers around many of the blood spaces. There was a decrease in the number and size of the basophilic cells.

In the cortex of the adrenal glands, many of the cells showed an altered staining reaction, and there was apparently a decreased endogenous secretion of epinephrine as the result of the injection of epinephrine hydrochloride.

It has been suggested that the action of epinephrine on certain animal tissues is in part a toxic or a degenerative one.



# AVITAMINOSIS

## III. PATHOLOGIC CHANGES IN TISSUES OF THE ALBINO RAT DURING EARLY STAGES OF VITAMIN A DEFICIENCY

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AND

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During the period from 1913 to 1922 it was generally assumed that deficiency of vitamin A produced only decline of body weight, frequently associated with xerophthalmia (first described by Osborne and Mendel<sup>1</sup> in 1913) and with respiratory disturbances (first observed by McCollum<sup>2</sup> in 1917). No other pathologic changes were recognized until 1922, when Mori<sup>3</sup> found cornification of the mucosa of the larynx and trachea and of the ducts of the submaxillary, sublingual and parotid glands. Mori referred to the change as keratinization. He attributed xerophthalmia to the drying of the epithelium due to the hypofunction of the lacrimal gland, the secretion of which keeps these epithelial surfaces moist.

In 1922, Evans and Bishop<sup>4</sup> reported peculiar changes in the estrual rhythm on a ration deficient in vitamin A. They stated:

It consists in the prolongation of the oestrus desquamative change in the vaginal epithelium, the smear consisting chiefly, if not exclusively, of the cornified cells which in normal individuals characterize the actual periods of oestrus and ovulation only, but which in the case of animals showing vitamin A deficiency occur throughout the entire period of acute deficiency.

Evans and Bishop reported that the preponderance of cornified cells in the vaginal epithelium was the most delicate and the first sign of vitamin A deficiency that they encountered, which became apparent long before cessation of growth took place.

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From the Department of Pathology, School of Medicine, University of Arkansas, Little Rock, and the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.

1. Osborne and Mendel: J. Biol. Chem. **16**:423, 1913.

2. McCollum: J. A. M. A. **68**:1379, 1917.

3. Mori: Bull. Johns Hopkins Hosp. **33**:357, 1922.

4. Evans and Bishop: J. Metab. Research **1**:10, 1922.

In 1925, Wolbach and Howe<sup>5</sup> confirmed the results of Mori and, in addition, found metaplastic changes in the pancreatic duct and renal pelvis. Metaplasia was found of the cylindric, cuboidal or transitional type of epithelium to the squamous, keratinizing type. They attributed these changes to vitamin A deficiency. They found the same changes in the salivary glands as Mori, but disagreed with the latter investigator in the claim that the secretions of these glands induced the metaplasia.

When Mori and Wolbach and Howe carried out their studies, vitamin A was not as yet differentiated into vitamins A and D, and the rations of these workers were therefore deficient in D as well as in A. Therefore, in 1927, Goldblatt and Benischek<sup>6</sup> reported their observations on diets deficient only in vitamin A, vitamin D having been furnished by irradiated cottonseed oil. They concluded that the metaplastic changes were as frequent and as great in rats on a diet deficient in vitamin A alone as in those on diets deficient in vitamins A and D. They also reported that, provided the vitamins were supplied, inanition, while producing atrophy of tissues, did not induce the metaplasia. They also noted the great frequency of abscesses at the base of the tongue.

In 1928, Green and Mellanby<sup>7</sup> reported that rats brought up on a diet deficient in vitamin A practically all died with infective or pyogenic lesions. In the control animals, receiving vitamin A, these lesions were absent. The presence of vitamin D did not prevent the resistance to infection. From their investigations they concluded that vitamin A is an anti-infective agent.

In 1929, Tyson and Smith<sup>8</sup> concluded that, in addition to metaplastic changes in various tissues, epithelial hyperplasia was striking in the tongue and renal pelvis, the hyperplasia in the latter overshadowing the keratinizing process. They stated that the metaplastic changes involved the following structures in order: the sublingual glands, the submaxillary glands and the epithelium of the renal pelvis as well as that of the trachea and bronchi. The tongue was regularly involved before xerophthalmia appeared. They also emphasized the existence of infection in vitamin A deficiency. They stated, "Infection is always present in the earliest stages and in late cases dominates the picture. No metaplastic activity has been seen without an accompanying infection, but infection has been observed in parts where metaplasia is absent."

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5. Wolbach and Howe: *Proc. Soc. Exper. Biol. & Med.* **22**:402, 1925; *J. Exper. Med.* **42**:402, 1925.

6. Goldblatt and Benischek: *J. Exper. Med.* **46**:699, 1927.

7. Green and Mellanby: *Brit. M. J.* **2**:691, 1928.

8. Tyson and Smith: *Am. J. Path.* **5**:57, 1929.

In 1923, Daniels<sup>9</sup> showed that in albino rats on diets deficient in vitamin A pyogenic infections developed in the sinuses and nasal cavities. These observations were later corroborated by Turner and his associates.<sup>10</sup>

Recently Green, Pindar, Davis and Mellanby<sup>11</sup> reported that vitamin A might be profitably used as a prophylactic agent against puerperal sepsis. Of a total of 550 pregnant women, 275 received the vitamin supplement, and 275 served as controls. "Of the 'vitamin treated' cases, 1.1 per cent, and of the controls, 4.7 per cent, developed the 'British Medical Association' standard of morbidity. The results classified on the basis of duration of pyrexia also suggested that the vitamin preparation increased the resistance of the puerperal women to infection."

The puzzling point in experimental research with reference to vitamin A is the criterion for the detection of the first signs of A avitaminosis. Some investigators claim that cessation of growth is the first expression of vitamin A deficiency, while other workers use the onset of ophthalmia as the standard. Evans and Bishop<sup>4</sup> claimed that prolongation of the cornified stage of the estrual cycle is the first manifestation of vitamin A depletion. Osborne and Mendel<sup>12</sup> found ocular symptoms in 69 of 136 rats on diets deficient in vitamin A, and Stephenson and Clark,<sup>13</sup> in 13 of 46 rats, while Sherman and Munsell<sup>14</sup> found an unmistakable disease of the eye in 85 per cent of their animals suffering from vitamin A deficiency. The latter investigators found distinct gatherings of pus in one or more of the glands near the base of the tongue in 76 per cent of the cases, and considered the base of the tongue as a characteristic seat of lesion in A avitaminosis. We found ophthalmia in 71 per cent of our pathologic animals. Recently, Honeywell, Dutcher and Ely<sup>15</sup> published the results of vitamin A investigations in which they employed two brands of yeast as a source of the vitamin B complex. With the use of one of these brands of yeast, the incidence of ophthalmia among the vitamin A deficient animals was only 20 per cent, but growth was poor. With the second brand of yeast, growth was good, but the incidence of ophthalmia was 100 per cent. They therefore suggested that "vitamin A may consist of two factors, one possessing anti-ophthalmic properties which tends to preserve normality of other epithelial tissues, and the other factor possessing the growth-stimulating properties usually ascribed to vitamin A."

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9. Daniels: *J. A. M. A.* **81**:828, 1923.

10. Turner: *Proc. Soc. Exper. Biol. & Med.* **26**:233, 1928. Shurly and Turner: *J. A. M. A.* **94**:539, 1930. Turner, Anderson and Loew: *J. Infect. Dis.* **46**:328, 1930. Turner and Loew: *J. Infect. Dis.* **49**:244, 1931.

11. Green, Pindar, Davis and Mellanby: *Brit. M. J.* **2**:595, 1931.

12. Osborne and Mendel: *J. A. M. A.* **76**:905, 1921

13. Stephenson and Clark: *Biochem. J.* **14**:502, 1920.

14. Sherman and Munsell: *J. Am. Chem. Soc.* **47**:1639, 1925.

15. Honeywell, Dutcher and Ely: *J. Nutrition* **3**:491, 1931.

The purpose of our investigation was to determine whether during the early stages of vitamin A deficiency, as evidenced by incipient signs of ophthalmia, a prolongation of the cornified stage during estrum or a cessation of growth, there were any significant metaplastic changes in the respiratory and urinary tracts, the posterior part of the tongue and the salivary glands. Microscopic studies were also made of the liver, adrenal glands, thyroid gland, hypophysis, thymus, bone marrow, genitalia, spleen, lymph nodes, pancreas, skeletal muscle, skin, larynx, urinary bladder, aorta, stomach, intestines including the cecum, and heart. The weight of the adrenal glands, heart, spleen, kidneys and liver were taken in forty-two pathologic animals and nineteen controls. Histologic changes were studied in fifty-three animals deficient in vitamin A and in twenty-four controls.

TABLE 1.—*Rations Deficient in Vitamin A Component*

Component	Ration 1749	Ration 1769
Casein (purified)*.....	20	20
Salts no. 185 †.....	4	4
N. W. yeast.....	10	10
Lard.....	2	1
Butter fat.....	..	1
Dextrin.....	64	64

\* Purified by several extractions with hot 95 per cent alcohol.

† McCollum and Simmonds: *J. Biol. Chem.* 33: 63, 1918.

#### VITAMIN A DEFICIENT DIETS USED; HISTOLOGIC TECHNIC.

The vitamin A deficient rations employed are listed in table 1. Ration 1749 was our standard vitamin A deficient diet. One per cent of butter fat was incorporated in ration 1769 so as to prevent the rapid onset of respiratory disturbances and thus to afford greater opportunity of detecting the incipient stages of A avitaminosis. Although the original plan was to deal only with early stages of this disease, advanced stages of vitamin A deficiency as characterized by marked ophthalmia were purposely allowed to develop in a number of animals so that a comparison might be made of the metaplastic changes in epithelial structures of various tissues during the early and later stages of A avitaminosis. As shown in table 2, the animals with marked lesions of the eyes showed a greater incidence of infection than the animals with incipient ophthalmia, although no marked differences had been observed in the extent of metaplasia.

The tissues were fixed immediately in Zenker's solution or in formaldehyde. After the Zenker fixation, they were stained by Giemsa's method according to the modification of Wolbach.<sup>16</sup> After fixation in formaldehyde, they were stained with hematoxylin and eosin.

16. Mallory and Wright: *Pathologic Technic*, ed. 8, Philadelphia, W. B. Saunders Company, 1924, p. 44.

## OBSERVATIONS

Most of our findings are summarized in tables 2 and 3. The remainder are presented in abstract form in the text that follows.

*Histologic Changes.*—The histologic changes were similar in most respects to those described by other workers, especially those reported by Wolbach and Howe.<sup>5</sup> In almost every rat there were metaplastic changes in the posterior portion of the tongue, with abscesses, dilatation of ducts, atrophy or fibrosis. Metaplasia was more common in the respiratory tract than in the ducts of the salivary glands or in the genito-urinary tract. After the posterior portion of the tongue, the upper respiratory tract was most frequently affected. This metaplasia was usually present in localized regions. Pneumonia occurred in five animals. The sublingual glands were involved more frequently than the submaxillary glands. The parotid glands were affected only occasionally. Atrophy of acini of the sublingual and submaxillary glands was noted; also atrophy of lining cells of the respiratory tract. Two of the tracheae were infected, including the glands.

**Urinary Tract:** It was common to see keratinization localized in the pelvis of the kidney. The urinary bladder was frequently involved in the same manner. Infection was noted five times.

**Liver:** Fibrosis about the portal spaces with infiltration by eosinophils and small lymphocytic cells was noted frequently. This change was also found in one of the controls, but it was not as marked as in the animals deficient in vitamin A.

**Adrenal Glands:** There were no noteworthy microscopic changes.

**Thyroid Gland:** A few of the acini appeared collapsed with lack of colloid, but this was seen in a few of the controls. In several thyroid glands, the acini were small, but this change was also present in the controls. In only one gland was definite atrophy of the cells lining the acini observed. No changes occurred in the parathyroid glands studied.

**Hypophysis:** No significant changes were found.

**Thymus:** In one slide there was an extremely large Hassall corpuscle with desquamation in the center not unlike an epithelial pearl. Some of the cells of Hassall's corpuscles appeared larger in the deficient animals than in the controls, but we do not care to make a positive statement with the examination of a comparatively small number of thymuses.

**Bone Marrow:** No noteworthy changes were found in the bone marrow except those changes present in acute infection.

**Female Genitalia:** The ovaries and tubes did not have any significant changes, but it would be necessary to examine a larger number to come

TABLE 2.—Pathologic Changes in Various Tissues of the Albino Rat in Vitamin A Deficiency

Animal	Duration of Experimental Period, Days	Changes in Weight Last 12-15 Days of Experiment, Gm.	Posterior Part of Tongue	Salivary Glands	Respiratory Tract	Urinary Tract	Other Pathologic Changes
♀ 1	40	+ 6	MDI*	AM	AMI	..	Uterus, M; spleen, A
♀ 2	43	+ 5	MDF	....	....	..	Liver, F about portal spaces; spleen, A
♀ 3	43	+ 8	MDI	A	AM	M	Liver, F about portal spaces; spleen, A
♂ 4	43	+ 9	MD	....	A	..	Liver, F about portal spaces; peribronchial infiltration
♂ 5	47	— 3	MDI	....	AM	M	Testes, A; spleen, A; heart, F and infiltration
♂ 6	47	+41	MD	AM	AM	..	Liver, F about portal spaces
♀ 7	46	+ 3	MDI	AM	AM	M	Liver, F about portal spaces; peribronchial infiltration
♀ 8	46	+ 8	MDI	M	AM	M	Liver, F about portal spaces; spleen, A
♂ 9	47	+ 3	MDI	M	AM	..	Uterus, MI; spleen, A; pancreas, A; peribronchial F
♀ 10	47	+ 3	MDI	AM	AM	..	Liver, fat
♀ 11	47	—25	MDI	MA	AM	M	Testes and spleen, A; peribronchial edema
♂ 12	47	—14	MDI	M	AM	M	
♂ 13	40	+ 3	F	....	M	M	
♀ 14	33	+30	MDF	....	AM	..	Spleen, A
♀ 15	40	+15	MF	AM	A	..	Spleen, A; peribronchial edema and infiltration
♀ 16	40	+14	MDIF	M	AM	M	
♀ 17	39	+15	MD	AM	AM	M	Liver, F about portal spaces
♀ 18	39	+ 4	MD	A	AMI	M	
♀ 19	39	+13	MD	....	AMI	M	
♀ 20	39	+10	MDF	....	M	M	Testes, A
♀ 21	34	+25	MDI	AMI	M	..	Spleen, A
♀ 22	34	+22	MDF	MA	....	..	Liver, F about portal spaces
♀ 23	34	+20	MF	A	M	..	Spleen, A; peribronchial infiltration
♀ 24	34	+15	MDI	Edema	A	..	Liver, focal necrosis; peribronchial infiltration
♀ 25	50	+16	MF	....	AM	..	Spleen, A; peribronchial infiltration
♀ 26	39	+ 6	F	M	MA	..	Spleen, A; heart, F
♀ 27	50	+ 5	MFI	A	M	..	Liver, F about portal spaces; spleen, A
♀ 28	39	+ 5	MDI	....	MA	..	Peribronchial infiltration
♀ 29	51	+ 5	MDF	MA	AMHP	..	Liver, F about portal spaces; spleen, A; bronchitis and pneumonia
♀ 30	47	+ 9	MDI	M	AM	M	Liver, F about portal spaces; peribronchial edema
♀ 31	47	— 1	MDIF	M	AMI	MI	Liver, F about portal spaces; spleen, A
♀ 32	38	+ 9	F	M	A	M	
♀ 33	36	+30	MD	A	....	..	Spleen, A; peribronchial infiltration
♀ 34	44	+11	MI	A	AM	..	Liver, focal necrosis and F about portal spaces
♀ 35	38	+ 1	F	....	MA	..	Heart, F; acute bronchitis and peribronchial infiltration
♀ 36	44	+10	M	M	AM	M	Liver, F about portal spaces; spleen, A
♀ 37	73†	—41	MDI	....	AMI	MI	Liver, fat; uterus, MI; heart, F; hair follicles of skin, A
♂ 38	62	—76	MDI	M	AM	MI	Liver, F about portal spaces; testes, A; peribronchial F
♀ 39	78†	—75	MDI	MA	MA	MI	Heart, F; pancreas, A
♂ 40	78	—84	MDI	M	AMI	M	Spleen and testes, A
♂ 41	78	—73	MDI	MDAI	M	MI	Testes and spleen, A

\* M indicates metaplasia; D, dilatation of ducts; I, infection; A, atrophy; F, fibrosis; B, bronchitis; P, pneumonia.

† Animal died.

to any conclusions. Two uteri showed marked cornification of the linings with infection. Another uterus showed cornification with marked infiltration by eosinophils in the submucosa.

Male Genitalia: Edema of the testes was seen occasionally. It was present beneath the capsule and extended slightly between the glands.

TABLE 2.—*Pathologic Changes in Various Tissues of the Albino Rat in Early Stages of Vitamin A Deficiency—Continued*

Animal	Duration of Experimental Period, Days	Changes in Weight Last 12-15 Days of Experiment, Gm.	Posterior Part of Tongue	Salivary Glands	Respiratory Tract	Urinary Tract	Other Pathologic Changes
♂ 42	78	-51	ID	MI	AMI	M	Testes, A; spleen, A; peribronchial edema; ducts of pancreas, M
♂ 43	109	-51		.	B	M	Testes, A
♀ 44	63	+11		.	..	..	
♀ 45	63	+ 5			P	M	
♀ 46	146	-13			....	M	Testes, A; peribronchial infiltration
♂ 47	145	-43			MI	..	Testes and spleen, A; peribronchial infiltration
♀ 48	52	+ 4			B	M	Liver, F about portal spaces; spleen A
♂ 49	50†	+ 5			P	M	Testes and spleen, A; liver, congestion
♂ 50	58	+ 2			APB	M	Testes and spleen, A
♂ 51	58	+ 7			....	M	Lungs, edema; spleen, A
♂ 52	58	-13			P	M	Heart, F; spleen, A; peribronchial infiltration
♂ 53	78	- 4			P	M	Testes and spleen, A; heart, F

TABLE 3.—*The Effect of Vitamin A Deficiency on Organ Weights of the Albino Rat*

Organ	Experimental Animals (42)		Control Animals (19)	
	Weight, Gm.	Percentage of Body Weight	Weight, Gm.	Percentage of Body Weight
Adrenal glands	0.0272	0.0185	0.0298	0.0176
Heart..	0.8326	0.5478	0.8158	0.4625
Spleen.	0.5005	0.3211	0.5733	0.3226
Kidneys	1.6379	1.0660	1.7047	0.9265
Liver..	6.1544	4.0741	7.6984	4.3519

In one animal there was extensive edema beneath the basement membrane. Atrophy of the tubules was common, either general or localized, with destruction of parenchymal cells. In one epididymis, the ducts were dilated with pus.

Spleen: Atrophy was usual.

Lymph Nodes: Those in the neck were larger than normal. There was slight hyperplasia, with phagocytic cells in abundance.

Heart: Fibrosis of the heart muscle was noted in two animals. Fragmentation was common, but this was present in the controls.

*Estrual Cycle in Early Stages of A Avitaminosis.*—The animals when started on the experiment were from 28 to 38 days of age and weighed from 41 to 84 Gm. The estrual cycles were followed daily in twenty pathologic animals and in twelve controls. At the time that the twenty pathologic rats were killed, owing to some early sign of vitamin A deficiency (as indicated by vaginal smear, incipient ophthalmia or cessation of growth), only nine of the females showed the persistence of the cornified stage of the estrual cycle. For instance, females 3 and 7, which were put to death at the first sign of slight ophthalmia, had metaplastic changes, also infection in the posterior portion of the tongue, before growth had ceased, yet these animals had a normal estrual picture. Female 31, which had no ophthalmia but had cessation of growth, metaplasia of the salivary glands and metaplasia with infection of the posterior part of the tongue and of the respiratory and urinary tracts, presented a normal estrual picture. The tongue, in addition, showed dilatation and fibrosis. Females 34 and 35 had incipient signs of ophthalmia and some metaplastic changes but a normal estrual cycle when they were killed, and female 34 was still growing. Female 11 not only had at the termination of the experiment metaplasia of the posterior part of the tongue, of the salivary glands and of the respiratory and urinary tracts, but also had lost considerable weight during the last two weeks of the experiment, and yet had a normal estrual cycle. From these results we conclude that a persistence of the cornified stage of the estrual cycle in the rat is not a positive index to the first signs of vitamin A deficiency, as claimed by Evans and Bishop.<sup>4</sup>

*Growth in Early Stages of A Avitaminosis.*—Neither is cessation of growth a reliable criterion of the first signs of vitamin A deficiency. It was found that thirty-eight of fifty-three animals, or 71 per cent of the animals deficient in vitamin A, showed metaplastic changes before decline of body weight or complete cessation of growth took place, as evidenced by weight records of the last two weeks of the experimental period. As a matter of fact, twelve of the thirty-eight animals still showed normal growth after manifesting metaplastic changes that were not detected in the controls.

It is interesting to note that female 45, which furnished external signs of incipient ophthalmia for a period of sixteen days before being put to death, was still actually gaining slightly in weight before the termination of the experiment, although having a cleancut case of pneumonia and metaplasia of the urinary tract. Also female 48 had bronchitis before cessation of growth took place, and female 49 died from pneumonia without any external signs of vitamin A deficiency. Male 53 had pneumonia accompanied by an insignificant loss of weight, and female 29 had pneumonia accompanied by metaplasia, atrophy and infection of the respiratory tract, metaplasia and atrophy of the salivary



glands and metaplasia, dilatation and fibrosis of the posterior part of the tongue, without any external signs of vitamin A deficiency.

An examination of table 3, showing the effect of vitamin A deficiency on organ weights, discloses no noteworthy changes in the weight of the adrenal glands, which we found hypertrophied in vitamin B deficiency.<sup>17</sup> There was no actual gain in the weights of the hearts of the pathologic animals, but when the heart weights were considered as percentages of body weights there was apparent a slight hypertrophy. The changes in weights of the kidneys, calculated as percentages of body weights, also indicated some hypertrophy in vitamin A deficiency. The livers showed some atrophy in this avitaminosis.

*Summary.*—It is clear from an analysis of our data that pathologic changes may take place in tissues as a result of vitamin A deficiency before any definite signs of external symptoms are apparent. We would therefore suggest from our experimental observations that, in vitamin A deficiency, metaplasia of epithelial structures in the respiratory tract of man possibly begins before definite external symptoms are evident, which later may be responsible for the respiratory disturbances, such as are found associated with the A avitaminosis—the common cold, bronchitis and bronchopneumonia.

#### REPORT OF A CASE OF A AVITAMINOSIS IN A CHILD

One of us (H. S. T.) recently studied the tissues of a boy, 13 months old, who evidently had been suffering from vitamin A deficiency.

The patient was admitted to the Children's Hospital in Little Rock, Ark., on Oct. 15, 1931, because of bilateral ophthalmitis with a left corneal ulcer, 5 mm. in diameter. He died on Oct. 29, 1931, with the clinical diagnosis of bronchopneumonia.

He had been fed breast milk exclusively from a mother who had been starved. He had been suffering undoubtedly from a lack of vitamin A in the diet, for, according to the careful experiments of Macy and others,<sup>18</sup> human milk does not contain a sufficient amount of this vitamin when the mother has not received it in the diet. The child's diet included, however, cod liver oil, as well as other sources of vitamin A, during the fourteen days in the hospital.

An autopsy limited to the neck, thorax and abdomen was made by Dr. A. F. DeGroat. The anatomic diagnosis was as follows: Corneal ulcer with perforation of the left eye; bronchopneumonia (bilateral); acute parenchymatous degeneration of the kidneys, liver and heart; acute splenic hyperplasia; lessened yellow material in the suprarenal cortices; slight emaciation; and hyperplasia of the splenic and mesenteric lymph nodes.

The microscopic examination showed: foci of subacute inflammation in the subpapillary layer of the corium, with occasional erosion of the lower layers of epithelium of the posterior part of the tongue; occasional metaplasia of the lining cells of ducts; occasional metaplasia of the lining cells of ducts of the sublingual

17. Sure, Thatcher and Walker: *Arch. Path.* **11**:413, 1931.

18. Macy, Outhouse, Graham and Long: *J. Biol. Chem.* **73**:175, 1927.

and submaxillary glands, with foci of subacute inflammation and destruction of acini in the latter; subacute inflammation of the submucosa of the larynx and trachea, with occasional metaplasia of the lining cells of tracheal ducts; subacute inflammation of the bronchial submucosa; acute bronchitis; bronchopneumonia; slight hyperplasia of the lining of the renal pelvis; congestion of the liver, and acute hyperplasia of the spleen.

There were no noteworthy changes in the heart, aorta, urinary bladder, ureters, intestines and testicles.

Xerophthalmia and keratomalacia in children on a diet deficient in vitamin A have often been reported. One of the latest of these reports is that by Block.<sup>19</sup> Unfortunately autopsies have not been studied to any extent with this point in view. The most convincing autopsy report is that by Wilson and DuBois.<sup>20</sup>

Our case was clinically and pathologically that of a child who had a diet deficient in vitamin A. Although the pathologic observations did not conform exactly to those in our rats, yet it was possible that the vitamin A in the cod liver oil and in the diet after admission to the hospital altered the original changes in the salivary glands and in the posterior part of the tongue. However, enough evidence was present in these tissues to place the lesions in the category of pathologic changes due to a diet deficient in vitamin A.

#### SUMMARY

Metaplastic changes in the posterior part of the tongue, in the salivary glands, and in the respiratory and urinary tracts were found during early stages of vitamin A deficiency in a large proportion of animals that made normal growth, some of which showed incipient ophthalmia or persistence of the cornified stage of estrum as the only sign of vitamin A deficiency, and others of which showed no external signs of A avitaminosis.

Bronchitis and pneumonia were also found in several animals that still made slight gains in weight before the termination of the experiment.

It is suggested that metaplasia of epithelial structures in the respiratory tract of man may be the precursor of the common cold, bronchitis and bronchopneumonia resulting from vitamin A deficiency, before any other external symptoms are detected by physical diagnosis.

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19. Block, C. E.: *Am. J. Dis. Child.* **42**:263, 1931.

20. Wilson, J. R., and DuBois, R. G.: *Am. J. Dis. Child.* **26**:432, 1923.

# RESISTANCE TO INSULIN

## THE RESPONSE TO INSULIN IN RABBITS DURING LEUKOCYTOSIS INDUCED BY SODIUM NUCLEINATE

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PHILADELPHIA

The increased dosage of insulin required to lower the blood sugar in many diabetic patients during an acute infection is a phenomenon of considerable theoretical interest for which no satisfactory explanation has yet been found. Infection not only appears to aggravate the diabetic state, causing marked loss of carbohydrate tolerance, but also often alters the response to insulin so that the dosage is out of proportion to the increased blood sugar incident to the infection. However, as Joslin<sup>1</sup> said, "At almost any moment during an infection recovery may begin and the need for insulin suddenly abate," and he warns against the danger of hypoglycemia at such a time. On the other hand, there are some cases recorded in which there is increased response to the usual dose of insulin during infection or gangrene.

When infection is superimposed on the diabetic state, the result is complex. If the response to insulin becomes decreased, how much of this is due to altered tolerance for carbohydrate incident to the infection with consequent greater requirement of insulin, and how much is due to actual inhibition of the action of insulin? The two processes cannot be separated in the diabetic patient. The phenomenon as seen in the diabetic patient might, however, be analyzed and simplified if one had more data on what effect infection per se, in the absence of diabetes, has on carbohydrate metabolism and the response of animals to insulin. There is some clinical evidence of disturbance in carbohydrate metabolism that may be incident to some infections in nondiabetic persons, as manifested by a slight rise in blood sugar values during fasting and by decreased tolerance for dextrose.

Alterations in carbohydrate metabolism occurring during infection might be explained in several ways:

1. Decreased utilization of carbohydrate might result from a depression in the activity of the pancreas. Although there is evidence that infection may cause anatomic injury to the islands of Langerhans, it seems inconceivable that an anatomic injury can account for the change

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1. Joslin, E. P.: *The Treatment of Diabetes Mellitus*, Philadelphia, Lea & Febiger, 1928, p. 711.

in response to insulin during infection in diabetic patients, as the restoration to previous conditions when the infection is removed is too rapid for anatomic regeneration to be possible.

2. The altered response to insulin during infection may be due to physiologic factors opposing the effect of insulin, such as a stimulation of the sympathetic nervous system and of the suprarenal and thyroid glands. Experimentally, intravenous injection of bacteria or of bacterial products into rabbits has been found by a number of investigators to cause a temporary elevation of the blood sugar level. In the case of *Bacillus coli* and *B. proteus*, the hyperglycemia in rabbits was found to be due to activity of the adrenal and thyroid glands (Geiger and Szirtes,<sup>2</sup> Evans and Zeckwer<sup>3</sup>). It is well known that after thyroidectomy a much smaller dose of insulin will cause convulsions than that which is required before thyroidectomy in the same animal. The antagonism between insulin and the adrenal glands has been demonstrated experimentally by a number of investigators. It is well established that when the blood sugar falls to a dangerously low level after administration of insulin, there is increased glycogenolytic activity. This effect antagonistic to the action of insulin depends not only on the degree of sympathetic and adrenal activity, but on how much glycogen is available for mobilization. Thus, if glycogen is abundant and readily mobilized, convulsions are inhibited. If there is little glycogen to be mobilized, or if mobilization is prevented by removing one adrenal gland and cutting the opposite splanchnic nerve, the animal goes into convulsions with an extremely small dose of insulin.

Reasoning then on this basis, if infection increased glycogenolytic activity, through the sympathetic nervous system, adrenal glands or thyroid gland, at first any insulin injected would be used up by the dextrose derived from hepatic glycogen, but if severe, prolonged infection might exhaust the glycogen store, there would be increased response to insulin if it were given in sufficiently large doses to lead to glycogenolysis. These changes being purely functional, prompt restoration to former conditions after infection has subsided could be more readily understood than if the disturbance in carbohydrate metabolism were due to anatomic injury to the islands of Langerhans. Probably involved in the whole matter is the increased catabolism of body tissues in sepsis.

Lawrence and Buckley<sup>4</sup> studied the response to insulin in rabbits before and after lethal doses of diphtheria toxin, and considered that there was a diminishing response to insulin after diphtheria toxemia was established. Unfortunately, it is hard to evaluate the results, as some

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2. Geiger, E., and Szirtes, L.: *Klin. Wchnschr.* 4:1912, 1925.

3. Evans, C. L., and Zeckwer, I. T.: *Brit. J. Exper. Path.* 8:280, 1927.

4. Lawrence, R. D., and Buckley, O. B.: *Brit. J. Exper. Path.* 8:58, 1927.

of the controls that had not received injections of diphtheria toxin showed hyperglycemia after insulin. One of these animals presented a blood sugar of 0.412 per cent three hours after the administration of insulin, with a drop in temperature; another rabbit that had been unaffected by the diphtheria toxin showed a blood sugar of 0.207 per cent in two hours after the administration of insulin and became limp, with a fall in temperature; in a footnote they state that three other rabbits showed similar phenomena. Since the blood was always withdrawn by cardiac puncture, a hemato-pericardium undoubtedly accounts for the rise in blood sugar and the fall in temperature. In my experience, a slight extravasation of blood into the pericardial sac of the rabbit causes respiratory embarrassment and hyperglycemia of asphyxial type.<sup>5</sup> If this hyperglycemia happened in the controls so frequently, the conclusions do not seem valid. Furthermore, results after diphtheria toxin has been given can scarcely be compared with the pyogenic infections frequently occurring in diabetic patients. Andrews and Schlegel<sup>5</sup> were unable to confirm Lawrence and Buckley's results, while Netzley<sup>6</sup> found insulin less effective when injected at the onset of intoxication by diphtheria toxin. Sweeney<sup>7</sup> found that diphtheria toxemia of rabbits did not cause a change in the action of injected insulin, but caused decreased tolerance of dextrose, and he suggested that the toxemia suppresses endogenous production of insulin.

3. A third possible explanation of the disturbance in carbohydrate metabolism during infection is that there is chemical inactivation of insulin, as by bacterial toxins or the enzymes of leukocytes and tissues.

Rosenthal and Behrendt<sup>8</sup> mixed human pus with insulin and then injected the mixture into rabbits. There was no effect of insulin. If the pus was first heated and then mixed with insulin, the injection resulted in an insulin effect. The insulin was injured by the proteolytic enzymes of the unheated pus, which is to be expected in view of the well known fact that insulin is rendered ineffective when mixed with proteolytic enzymes. They found that rabbit pus had slight effect in comparison with human pus. Similar experiments were carried out by Karelitz, Leader and Cohen,<sup>9</sup> who found that when insulin was incubated with pus from a patient with empyema or with cells of myeloid leukemia or with the plasma of patients with infection, the insulin effect was inhibited, unless the substance was heated before being mixed with insulin. Andrews and Schlegel<sup>5</sup> found that when necrotic material was

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5. Andrews, E., and Schlegel, K. W.: *Arch. Int. Med.* **40**:637, 1927.

6. Netzley, R. E.: *Am. J. Dis. Child.* **37**:511, 1928.

7. Sweeney, J. S.: *Arch. Int. Med.* **41**:420, 1928.

8. Rosenthal, F., and Behrendt, R.: *Ztschr. f. d. ges. exper. Med.* **53**:562, 1926.

9. Karelitz, S.; Leader, S. D., and Cohen, P.: *Arch. Int. Med.* **45**:690, 1930.

injected simultaneously with insulin into a normal animal, no inhibition of insulin occurred. In this case, there probably had not been time for *in vitro* destruction of insulin.

Whether such wholesale destruction of insulin could be caused *in vivo* by the enzymes of circulating leukocytes as occurs when pus and insulin are mixed *in vitro* is unanswered by these experiments. Perhaps a method that approaches better the actual conditions is that of Depisch and Hasenöhr, <sup>10</sup> who injected a convulsant dose of insulin after injecting the serum of a diabetic person who was insulin-resistant owing to infection, and found that convulsions did not result. This was controlled by noting the usual effect of insulin when the same dose was injected with the serum of a diabetic person who was responsive to insulin. These experiments indicate that enzymes may be circulating in the blood in sufficient quantities to inhibit injected insulin. However, it is stated that the blood from three other insulin-resistant persons did not give corresponding results.

Karelitz, Leader and Cohen <sup>9</sup> injected typhoid or staphylococcus vaccine into rabbits to simulate the effect of infection, and found that some of the animals showed a moderately decreased insulin effect, and they interpreted this as evidence that some substance chemically inhibits the action of insulin. Harris, Ringer and Lasker <sup>11</sup> incubated cultures of streptococci with insulin, then injected the mixture into mice, and obtained the same effect as with insulin alone. Similarly Andrews and Schlegel, <sup>5</sup> mixing dead staphylococci with insulin, obtained an insulin effect. The explanation probably is that these bacteria have not destroyed the insulin by proteolytic enzymes.

Buckley <sup>12</sup> injected trypsin into rabbits and then studied the response to insulin, and concluded tentatively that injections of trypsin lessen the response to insulin. Here, again, the blood was withdrawn by cardiac punctures, and the effect of this, previously discussed, is manifested in two of the rabbits, for which rather high blood sugar values were recorded before trypsin was injected.

Whether the enzymes of circulating leukocytes may neutralize insulin *in vivo* is an important point to determine. A number of foreign investigators have been concerned with this problem, and a study has been made of the effect of insulin during leukocytosis induced by sodium nucleinate, with variable results. No data have been found in the literature correlating leukocyte counts of patients with resistance to insulin during infections, and no data have been found of any difference in response to insulin in the rare cases in which diabetes and leukemia

10. Depisch, F., and Hasenöhr, R.: *Ztschr. f. d. ges. exper. Med.* **58**:110, 1928.

11. Harris, M. M.; Ringer, A. I., and Lasker, M.: *Arch. Path.* **4**:546, 1927.

12. Buckley, O. B.: *Brit. J. Exper. Path.* **12**:13, 1931.

occur in the same patient. Rosenthal and Behrendt<sup>5</sup> studied the reaction to insulin in three nondiabetic patients with infections, but the data show that these patients had no marked leukocytosis. In two of these, patients with empyema, there was a very slight response to insulin, while in the third, a patient with pyonephrosis, a marked lowering of the blood sugar level occurred. It is not known what the response to the same amount of insulin was in the same patients in the absence of infection.

#### EXPERIMENTAL PROCEDURES

Experiments were carried out in an attempt to study what factors may be involved in the altered response to insulin during acute infection. The problem was narrowed down to the question of whether leukocytosis induced by a chemical agent, such as sodium nucleinate, which has been used by other investigators for similar purposes, rather than by a bacterial agent, modifies the response to insulin in rabbits by chemical or by physiologic antagonisms.

In the first experiments, nonconvulsive doses of insulin were used, but the results showed that the drop in blood sugar from a normal level to a level above the point at which convulsions would occur was so slight and so variable that no clearcut comparison could be made between normal and experimental conditions. Larger doses were therefore used, and convulsions were taken as the criterion for insulin action. Preliminary experiments<sup>13</sup> indicating that leukocytosis may in a certain number of cases inhibit the action of insulin were followed by the more complete experiments reported in this paper.

Since the response of individual rabbits to insulin varies considerably, and varies in the same rabbit at different times, instead of a group of control rabbits being compared with a group of experienced animals, each animal was its own control; that is, the response to insulin was determined one or, more often, several times under standard conditions, then the same animal was given an intravenous injection of sodium nucleinate to produce leukocytosis, and the response to insulin under these conditions was compared with the previous response under normal conditions. In most cases, the animal was allowed to recover from the experimental condition, and then at a later date was again tested for a normal response, in order to reduce the possibility that the animal had changed in response to insulin merely because of often repeated doses.

The intravenous injection of sodium nucleinate in doses of 0.1 Gm. results in transient leukopenia followed by leukocytosis, which persists for many hours. Repeated doses are well tolerated, and leukocytosis induced by this chemical agent is more easily controlled than a bacterial infection and permits a return to normal conditions for comparison. A fast of a number of hours, stated in the table, preceded the injection of insulin. This period was not always exactly the same, but in a herbivorous animal, in which the stomach is full even after a long fast, the period of fast need not be controlled as rigidly as in other animals, as shown by Scott,<sup>14</sup> who found that the maximum precision is obtained in rabbits after about ten hours' inanition, and that the blood sugar remains steady for the following

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13. Zeckwer, I. T.: *Arch. Path.* 9:1297, 1930.

14. Scott, E. L.: *Arch. Int. Med.* 43:393, 1929.

fourteen hours. Injections of insulin were given at appropriate intervals so as to insure that regular feeding was not interfered with between consecutive injections.

Determinations of the preliminary blood sugar levels were made by the Hagedorn and Jensen method, requiring 0.1 cc. of blood, and the occurrence of hypoglycemic convulsions after a small convulsive dose of insulin was taken as the criterion of insulin action. Many of the blood sugar determinations were made by Mr. A. J. Abbott and Mr. Fred W. Van Buskirk, Jr. As soon as the convulsions occurred, dextrose was given intraperitoneally or intravenously to prevent further loss of hepatic glycogen and to save the animal for later experiments. Leukocyte counts were made immediately before the injections of insulin on blood flowing freely from a prick of a vessel of the ear.

The table gives four examples of the results. For the sake of brevity, the results of all the injections of insulin are not recorded, those omitted being results similar to those recorded or the results of injection of insulin in doses far above or far below the approximate minimal convulsive dose.

#### RESULTS

*Response to Insulin During Leukocytosis Induced by Injections of Sodium Nucleinate in Intact Rabbits.*—In this group twelve intact rabbits were studied, and two effects were noted:

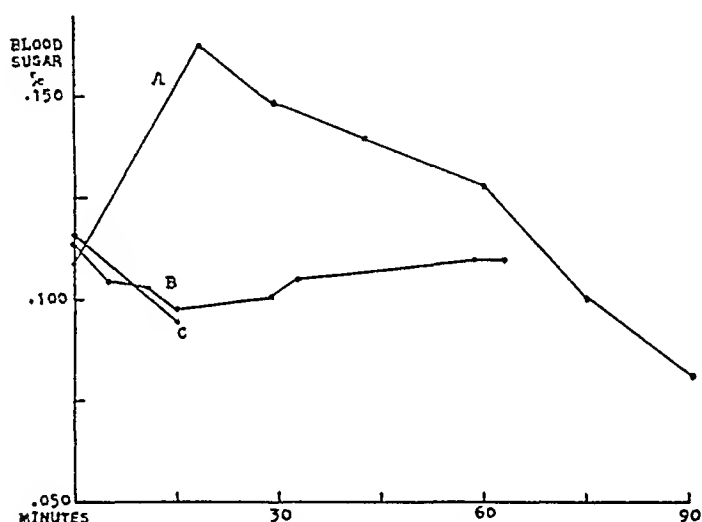
1. In three rabbits (29, 33 and 51), definite inhibition of the action of insulin did not occur. Instead, the response to insulin appeared in most instances to be greater after the injection of sodium nucleinate than under normal conditions. In rabbit 29, when blood sugar values were determined, it was found that twice there was a low preliminary level some hours after the injection of sodium nucleinate and just before the insulin was injected. In the other two rabbits (33 and 51), when sugar determinations were made, the level was not lowered, but in rabbits 31, 38 and 40 there was a low blood sugar level after the injection of sodium nucleinate. This indicated the need of following the blood sugar for a number of hours after injection of sodium nucleinate. Immediately after injecting sodium nucleinate the blood sugar rose; it reached its highest point in about fifteen minutes and dropped to below normal in about two hours, a type of curve suggesting a sudden release of glycogen from the liver by activity of the sympathetic nervous system or of the adrenal glands (chart 1). Twenty-four hours later the blood sugar was either at a normal level or at a lower level. The low blood sugar levels found in certain rabbits occurred only when more than one injection had been given. It is doubtful if a single injection would be sufficient to deplete appreciably the store of glycogen. In two rabbits in which one adrenal gland had been removed and the opposite splanchnic nerve cut, no rise in blood sugar occurred after the injection of sodium nucleinate, but instead a slight drop, proving that when hyperglycemia



results from sodium nucleinate in the intact animal it is because glycogen is discharged from the liver through sympathetic-adrenal activity (chart).

2. Among the other nine rabbits were three (31, 32 and 50) that showed doubtful inhibition of convulsions during leukocytosis. In rabbits 31 and 32, two out of three times in each animal under normal conditions insulin resulted in convulsions, while both times after the injection of sodium nucleinate, convulsions did not occur with the same dosage. Rabbit 50 showed inhibition once and failed to show it a second time.

The other six (35, 37, 38, 49, 53 and 54) showed apparent inhibition of convulsions, provided the leukocyte count was high after the injection of sodium nucleinate, and provided the dose of insulin was not much



Blood sugar values after intravenous injection of 0.1 Gm. of sodium nucleinate: A, in intact rabbit 37; B, in rabbit 39 after right adrenal gland had been excised and left splanchnic nerve cut; C, in rabbit 49 after left adrenal gland had been excised and right splanchnic nerve cut.

above the minimal convulsive dose. In those cases in this group (31 and 38) in which blood sugar values were low after the injection of sodium nucleinate, inhibition occurred in spite of the low blood sugar levels at the time of the injection of insulin. It has been shown that each injection of sodium nucleinate apparently mobilizes glycogen from the liver, and that if these injections are repeated at short intervals the animal has less glycogen available for counteracting the effect of insulin, and yet, in spite of this loss of glycogen, insulin convulsions may be prevented. Blood sugar values taken at the time of convulsions were in most instances lower than those taken when convulsions were inhibited, the blood in the latter case being obtained at the same interval

# *Experimental Alteration of Responses to Insulin in Rabbits*

Rabbit	Date	Body Weight, Kg.	Hours of Fast	Experimental Condition	Blood Sugar Percentage Before Injection of Insulin	Units of Insulin Injected	White Blood Cell Count	Convulsions	Time of Convulsions, Min. After Injection of Insulin	Comment
R 29	5/10/29	1.84	22	Normal	0.111	2	4,800	+	85	No inhibition
	6/ 5/29	....	16	17 hr. after 1 Gm. sodium nucleinate	0.073	2	27,000	+	75	Nucleinate increased effectiveness of insulin
	6/11/29	1.64	21	20 hr. after 0.1 Gm. sodium nucleinate	....	2	91,600	+	70	
	6/28/29	....	24	Normal	0.111	1	6,100	0	...	
	7/ 9/29	1.82	24	18 hr. after 0.1 Gm. sodium nucleinate	0.063-0.086	1	21,000	+	90	
R 35	10/29/29	....	21	Normal	....	1	14,000	+	110	Before operation, inhibition with 1 unit when leukocytes were high, but not with 2 units
	11/12/29	2.14	17	Normal	0.108	0.5	6,000	0	...	
	11/16/29	....	19	19 hr. after 0.1 Gm. sodium nucleinate	0.091	2	21,900	+	50	
	11/19/29	2.04	20	20 hr. after 0.1 Gm. sodium nucleinate	0.124	1	13,400	+	80	
	11/26/29	2.00	18	18 hr. after 0.1 Gm. sodium nucleinate	0.083	1	20,600	0	...	
	12/ 3/29	2.06	20	20 hr. after 0.1 Gm. sodium nucleinate	0.107	1	27,000	0	...	
	12/ 5/29	....	18	18 hr. after 0.1 Gm. sodium nucleinate	....	1	11,800	+	115	
	12/19/29	2.24	..	Left adrenal excised, right splanchnic nerve cut	....	...	...	...	...	
	12/31/29	2.16	20	12 days after operation	0.101	0.5	16,400	+	80	After operation, inhibition in spite of low blood sugar
	1/ 3/30	1.94	17	17 hr. after 0.15 Gm. sodium nucleinate	0.086	0.5	77,800	0	...	
R 40	9/24/30	2.00	21	Normal	0.103	2	13,000	+	105	
	9/30/30	1.94	21	Normal	0.116	2	15,000	+	120	
	10/22/30	2.14	20	21 hr. after 0.15 Gm. sodium nucleinate	0.113	2	22,600	+	140	
	11/ 5/30	....	21	21 hr. after 0.15 Gm. sodium nucleinate	0.115	2	74,000	0	...	
	11/11/30	2.40	20	Normal	0.076	2	19,400	+	165	Inhibition, when leukocytes were high, with dose larger than minimal convulsive
	11/25/30	....	19	19 hr. after 0.15 Gm. sodium nucleinate	0.100	2	36,800	0	...	
	12/ 5/30	2.62	19	Normal	....	2	15,700	+	180	
	12/10/30	2.60	16	Normal	0.094	2.25	12,400	+	150	
	12/11/30	2.60	16	16 hr. after 0.15 Gm. sodium nucleinate	....	2.25	52,000	0	...	
	5/ 5/31	....	..	Thyroidectomy	....	2	....	0	...	After thyroidectomy, inhibition with dose larger than minimal convulsive
R 67	6/17/31	2.04	18	Normal	....	2.5	....	+	165	
	6/26/31	2.08	18	Normal	....	2.75	15,000	0	...	
	6/30/31	2.04	18	18 hr. after 0.1 Gm. sodium nucleinate	....	3	110,600	0	...	
	7/ 9/31	2.06	20	18 hr. after 0.1 Gm. sodium nucleinate	0.099	2.75	10,600	+	135	
	7/11/31	1.98	17	Normal	0.115	2.75	9,800	+	...	

of time after the injection of insulin as that at which the rabbit had previously shown convulsions.

This inhibition of insulin convulsions may have two explanations: 1. Glycogenolytic activity may be increased by sodium nucleinate even after the initial glycogenolysis, thus offering a physiologic antagonism to the hypoglycemic effect of insulin. 2. The circulating leukocytes may inactivate insulin, probably by their proteolytic enzymes. To gain data supporting or controverting the postulates just outlined, further experiments were carried out.

*Effect of Sodium Nucleinate on Action of Insulin at Time When Leukocytosis Fails to Occur.*—When sodium nucleinate is repeatedly injected, the bone marrow becomes more or less depleted of myeloid elements, so that a marked rise in circulating leukocytes no longer occurs (Doan, Zervas, Warren and Ames;<sup>15</sup> Zeckwer<sup>16</sup>). If the response to insulin after the injection of sodium nucleinate in such an animal with no leukocytosis is compared with that in the same animal under normal conditions and with that after previous injections of sodium nucleinate from which marked leukocytosis resulted, any alteration in the response might be due to the numbers of leukocytes and not to the sodium nucleinate per se.

Rabbits 37 and 38 showed that apparent inhibition of the action of insulin occurred when the leukocytosis was marked, but inhibition failed to occur when leukocytosis was not present (see table). In rabbit 37, the leukocyte count was only 7,400 seventeen hours after the third injection of sodium nucleinate, and this time 2 units caused convulsions, while a similar dose had failed to cause convulsions on two previous occasions when the leukocyte counts were 22,800 and 32,000. In rabbit 38, when the leukocyte count after a repeated injection of sodium nucleinate was only 13,000, insulin convulsions were not inhibited, but after the bone marrow was rested, subsequent injections of sodium nucleinate resulting in a leukocytosis of 20,600 and 27,000 inhibited the same dose of insulin, while after a still later injection of sodium nucleinate resulting in a leukocyte count of only 11,800, convulsions occurred.

Other rabbits showed the same phenomenon. For instance, rabbit 49 showed inhibition of convulsions when the leukocyte counts were very high, but not when leukocytosis was only moderate.

These results appeared to be evidence that when the action of insulin had been inhibited, this was due to the leukocytes, not to the effect of sodium nucleinate per se. Nevertheless, since sodium nucleinate had

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15. Doan, C. A.; Zervas, L. G.; Warren, S., and Ames, O.: J. Exper. Med. **47**:403, 1928.

16. Zeckwer, I. T.: Arch. Path. **7**:1012, 1929.

been definitely shown to mobilize glycogen, both factors might be concerned. Accordingly, it now became necessary to see what effect eliminating the physiologic factors responsible for glycogenolysis had on the response to insulin after the injection of sodium nucleinate.

*Effect on Response to Insulin During Sodium Nucleinate Leukocytosis of Inhibiting Glycogenolysis by Removal of One Adrenal Gland and Cutting of Opposite Splanchnic Nerve.*—In three rabbits one adrenal gland was removed and the opposite splanchnic nerve cut under aseptic conditions, and recovery from the immediate effect of the operation was permitted by a rest of a number of days before the response to insulin was studied. It has been shown by a number of investigators that in such animals the minimal convulsive dose of insulin is much smaller than in intact animals, as the action of insulin is opposed by less glycogenolysis. When a small convulsive dosage was determined after operation, sodium nucleinate was injected and the response to insulin tested, as before, in these animals that had been operated on.

In such animals any effect that sodium nucleinate might have in mobilizing glycogen and in that way inhibiting insulin was prevented, as previous experience (Evans and Zeckwer<sup>2</sup>) had convinced us that such a procedure was functionally effective in preventing glycogenolysis, in accordance with the findings of a number of previous workers. If any inhibition of the action of insulin should then occur, it would be due to the leukocytosis alone.

A functionally successful operation in these animals was tested in several ways: 1. A series of blood sugar determinations was carried out immediately after the injection of sodium nucleinate, and it was found that the hyperglycemia that occurs in the intact animal no longer occurred, but, instead, a fall. Rabbits 39 and 40 were tested this way. 2. The minimal convulsive dose of insulin before operation was compared with that after operation, and it was found that a much smaller dose was effective after operation (rabbits 38 and 39). There was additional evidence in the manner in which the animals reacted after convulsions occurred. In the normal rabbit, intraperitoneal injection of dextrose resulted in an almost instantaneous recovery from convulsions, the animal quickly jumping to its feet and running away as though nothing had happened. The animals operated on were severely prostrated and showed a slow recovery after receiving dextrose, as one would expect if the compensatory release of glycogen in emergency could no longer occur.

The results were as follows: In rabbit 39, seven days after operation, one-half unit of insulin resulted in convulsions. Six days later sodium nucleinate was injected, but the leukocyte count was only 6,000 when insulin was injected, and the injection of the insulin again resulted in

convulsions. Fifteen days later a larger dose of sodium nucleinate was given, resulting in a leukocytosis of 28,000, and with the same dose of insulin convulsions were prevented. After an interval of a number of days, insulin in the same dose again caused convulsions when the level of the leukocytes was low.

In rabbit 40, inhibition of the action of 1 unit of insulin was not obtained after operation during leukocytosis. This rabbit had not been tested for inhibition before operation.

In rabbit 38, in which before operation inhibition had been shown, after operation one-half unit caused convulsions, but when leukocytosis was produced by sodium nucleinate, convulsions were inhibited.

In rabbit 41, both splanchnic nerves had been cut, but neither adrenal gland had been removed. One unit of insulin caused convulsions before sodium nucleinate was injected, but failed to cause convulsions during the leukocytosis following the injection of sodium nucleinate.

It was concluded from these experiments that, since inhibition of convulsions could occur in animals in which glycogenolysis was prevented by operation, the inhibition of convulsions was not because sodium nucleinate favored a compensating glycogenolysis.

*Effect of Thyroidectomy on Response to Insulin During Sodium Nucleinate Leukocytosis.*—In view of the influence of the thyroid gland on glycogenolysis, discussed in previous work,<sup>17</sup> three thyroidectomized rabbits were studied. Rabbit 54 had shown apparent inhibition of insulin convulsions before thyroidectomy, and after thyroidectomy it showed inhibition during sodium nucleinate leukocytosis with the previous convulsive dose, but not with a dose larger than that. Rabbits 67 and 69, the response of which to insulin had not been tested before thyroidectomy, showed inhibition after thyroidectomy with doses one-half unit higher than the previous convulsive dose, provided the leukocyte count was high.

#### COMMENT

One possible source of error must always be considered in experiments of this type, and that is the fluctuations in response to insulin which rabbits show under standard conditions. For instance, a rabbit may tolerate without convulsions ascending doses of insulin and finally succumb to, let us say,  $2\frac{1}{2}$  units. Having once had convulsions, it may be more sensitive, and fits may occur with 2 or  $1\frac{1}{2}$  units. This spontaneous fluctuation is commonly neglected in many experiments having to do with the effect of insulin in rabbits reported in the literature. It was for this reason that the rabbits were studied under normal conditions, then during leukocytosis, then again under normal

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17. Zeckwer, I. T.: Am. J. Path. 7:548, 1931.

conditions, and then, when possible, again during leukocytosis, in order to reduce as far as possible the likelihood that the results merely coincided with spontaneous fluctuations in response to insulin. However, all possibility that the results noted were influenced by spontaneous fluctuations cannot be ruled out.

These experiments, then, give suggestive but not conclusive evidence that the convulsions following injection of insulin in rabbits may in a certain number of cases be inhibited during leukocytosis induced by sodium nucleinate, but the inconstancy of the results makes definite conclusions impossible. Although after the injection of sodium nucleinate many factors other than leukocytosis may enter into the picture, such as changes in the metabolic rate, yet leukocytosis would seem to be the important factor, as the apparent inhibition, when it occurs, is roughly proportional to the degree of leukocytosis. Since leukocytes destroy insulin *in vitro*, it seems possible that the enzymes of circulating leukocytes may destroy insulin *in vivo*.

These experiments throw no clear light on the resistance to insulin in diabetic patients with infections. The apparent inhibition, when obtained in these experiments, was only slight in comparison with the resistance shown in man. However, the proteolytic activity of rabbit leukocytes is much less than that of human leukocytes. It would therefore be of considerable interest to have data on leukocyte counts of patients showing resistance to insulin during acute infections. In man, if leukocytic enzymes play a rôle in this resistance to insulin, there are probably other factors as well.

The use of convulsive doses makes the results more concrete than when a comparison is made of the falls in the blood sugar after nonconvulsive doses. The results with a convulsive dose, however, cannot be compared with the effects of the dosage used in diabetic patients. The use of convulsive doses is a test of the glycogen store, and of the animal's ability to mobilize this store when a dangerous level of blood sugar is reached, while with the dosage of insulin properly used in man, glycogenolysis probably does not occur.

These observations apply only to rabbits. Other animals may behave differently. For instance, the injection of "coli toxin" (Geiger<sup>18</sup>) caused hyperglycemia in rabbits and hypoglycemia in dogs. In studies now in progress, in the one depancreatized dog so far studied, bacterial infection lessened the drop of blood sugar with a small, nonconvulsive dose of insulin, but during leukocytosis of the same degree induced by sodium nucleinate the same dose of insulin was not inhibited. Since so much of the experimental work on insulin has been done on rabbits,

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18. Geiger, E.: *Arch. f. exper. Path. u. Pharmakol.* **121**:67, 1927.

the present experiments are reported in spite of the fact that there is no justification for applying these results to the problem of the response to insulin in other animals or in man.

#### SUMMARY

Seventeen rabbits were studied for the response to insulin during leukocytosis induced by intravenous injection of sodium nucleinate. The results were variable. In three of twelve intact animals, no inhibition of insulin convulsions occurred during leukocytosis; three showed doubtful alterations in response, and six showed apparent inhibition of insulin convulsions, provided that there was a high degree of leukocytosis, and provided that a small convulsive dose of insulin was used; but the possibility of spontaneous fluctuation in the response to insulin cannot be ruled out, although the experimental methods employed reduced this possibility. Apparent inhibition of insulin convulsions after injections of sodium nucleinate may occur after glycogenolytic activity is reduced by (1) removing one adrenal gland and cutting the opposite splanchnic nerve (two of three rabbits), and by (2) thyroidectomy (all of three rabbits).

Sodium nucleinate injected intravenously causes an immediate hyperglycemia, which is due to glycogenolysis, for it is prevented by previous removal of one adrenal gland and cutting of the opposite splanchnic nerve.

These results do not elucidate the problem of the altered response to insulin in diabetic patients with acute infection, for reasons given in the text.

# General Review

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## THE ORIGIN AND ANTIQUITY OF SYPHILIS: THE EVIDENCE FROM DISEASED BONES

A REVIEW, WITH SOME NEW MATERIAL FROM AMERICA

HERBERT U. WILLIAMS, M.D.

BUFFALO

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Evidence for the existence of syphilis in ancient times may conceivably be derived from written documents or from pictures made by artists of the lesions of the disease or from human remains showing its effects. In the last case, bones must be the principal, if not the only, structures available for study. In this article, I propose reviewing all the ancient bones alleged to be syphilitic of which I can find descriptions in the literature. For the controversy over the testimony of ancient documents, begun four centuries ago, still goes on, and portrayals of disease by ancient artists are few and indecisive. But the industry of archeologists in recent years has brought to light a wealth of material that makes chronology more exact, and that includes some important skeletal remains. I am therefore able to describe some specimens not previously reported.

### THE ARCHEOLOGIC PROBLEM

It has often been stated that, for the purposes of such an inquiry as this, one must have proof that a bone is ancient and that it is syphilitic. It is owing to a difference of opinion as to what constitutes proof that the controversy continues.

Now if one could open an intact tomb, inscribed 1000 A.D. or the equivalent of 1000 B.C., containing a skeleton that all pathologists agreed must be syphilitic and could be nothing else, that would be convincing; almost too convincing.

Most of the royal tombs in Egypt were plundered and the mummies in them more or less disturbed. The tomb of King Tutankhamen was the exception that made its discovery famous (Smith and W. R. Dawson). Graves in ancient Greece were robbed or used for burials by later peoples (intrusive burials). The same practice was prevalent among many peoples and in many parts of the world, both in ancient and in modern times.

Some of the most famous of early human remains were found as scattered fragments no longer in their original position. Mr. Charles Dawson recovered parts of the Piltdown skull from spoil heaps where they had been thrown by workmen; other parts were taken from the gravel pit in their original position, considerable times intervening between the different finds. The Neandertal skull and bones were dug out by laborers from a cave and thrown aside, to be recovered later in part by Dr. Fuhlrott. The Gibraltar skull lay for several years in the museum of the Royal College of Surgeons, unhonored and unnoticed; its history was defective, and apparently no record was preserved of the precise circumstances under which it was found. Nevertheless, no archeologist has seemed to doubt the validity of any of these priceless specimens (Hrdlicka, 1914; MacCurdy, 1924).

An investigator working in a period when, or in a region where, there was no written language must enjoy a comfortable sense of security in dating his find if he can demonstrate successive strata of later dates lying above it; as when he discovers neolithic material overlaid by material characteristic of the cultures of the Bronze Age and of historic times. Such sites occur in western Europe. But in many cases there is no such stratification, and he calls his find neolithic because it contains objects that in other places have been proved by stratification to be characteristic of neolithic culture, as polished stone implements, exquisitely flaked flint tools, pottery and certain styles of ornaments; at the same time, it must not contain objects characteristic of later cultures. The plan is the same as that used by geologists when they determine the age of an outcrop of rock by the fossils that it contains. The dates of a large part of the objects displayed in museums of archeology are based on such evidence. All the specimens of bones alleged to be prehistoric and also syphilitic are dated by similar evidence. None of them is accompanied by testimony of any written language; with none of them is there any important assistance from stratified deposits overlying them.<sup>1</sup> Their antiquity is inferred from the type of burial and from the associated pottery, implements and ornaments, and from the absence of objects of later periods. The circumstances surrounding each of the alleged cases and the validity of the evidence are considered hereafter separately. As in criminal trials, a sufficient number of probabilities, all pointing in one direction, may make a powerful case.

Most of the specimens of bones supposed to be ancient and syphilitic reported in recent years have been found in America, so that a brief discussion of the peculiar features presented by American archeology will be appropriate at this point.

In an important paper on prehistoric syphilis by Virchow in 1896, which has often been quoted, it was pointed out that the American Indians continued to use the implements and ornaments of their native culture long after the arrival of the white race. A grave containing the objects characteristic of Indian culture and no objects derived from the whites was not necessarily ancient. Also, those examples of stratification that were quite frequent in Europe were rare in both North and South America.

However, much progress has been made since 1896. Archeologists have been active in studying American problems. One can now be certain in many cases that cultural material and bones are pre-Columbian.

It seems that the Indians of practically the whole of North and South America had a neolithic culture; in Mexico, Central America and Peru, bronze was beginning to be used, but its use was not general.

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1. The specimens from Pecos, N. M., may be an exception.

The degree of development of this neolithic culture varies immensely in different parts of America, and may sometimes be used roughly in dating ancient remains. A simple illustration from the state of New York serves as an example.

The first white explorers found most of what is now the state of New York occupied by the Iroquois family of Indians, but even in the middle of the Iroquois region there were clear evidences of an earlier occupation by people having the culture characteristic of Algonkian Indians (Parker). An Algonkian grave occurring in the Iroquois area therefore antedated the arrival of white men.<sup>2</sup>

In the Ohio and Mississippi valleys were the famous works of the Indians called Mound Builders. Most of the mounds were ancient and prehistoric, but some of them were erected by modern Indians. Some contained objects obtained from white men. A relic coming from one of the mounds was not necessarily prehistoric. Certain bones, strongly suspected of being syphilitic, were derived from burial grounds of the Mound Builders.

Archeologists in this field have gone far toward classifying the cultures of the mounds. For instance, that found in the Hopewell group of mounds is a well defined, ancient and prehistoric culture recognizable over a wide area (Mills, Shetrone). Two other cultures, the Adena and Fort Ancient cultures, also appear to have been prehistoric. Furthermore, Shetrone<sup>3</sup> expressed the belief that only a small number of mounds have been built during the historic period, although intrusive burials, recognizable to an expert as such, are not infrequent on the surfaces of these mounds.

The Indians of New Mexico and the adjoining states built impressive structures of stone, often high up under overhanging cliffs (Cliff Dwellers). In open places, they erected the great communal dwellings, like the modern apartment house, called pueblos (the Indians are also called Pueblos). It has been shown that the Pueblos were preceded by a people known as Basket Makers, who had a primitive culture and to whom stone houses and pottery were unknown. Through the efforts of a large number of archeologists,<sup>4</sup> a succession of cultures has been

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2. Dr. Parker recently encountered a curious and interesting find in excavating an Iroquois grave in the Cayuga Lake region; the grave contained a coin of the Roman colony in Egypt. The coin, of course, indicated that the burial was modern and not ancient, and that the Indian obtained it from some European missionary or trader.

3. I have been fortunate, as an amateur, in being able to watch Shetrone's technic in the field. As Wissler put it, Shetrone "dissects" a mound in the way that a pathologist dissects a body.

4. The well known and honored American pathologist, T. Mitchell Prudden, who was also an enthusiastic archeologist, was prominent in helping to solve the puzzles presented by the ruins of the Southwest.

discovered: Basket Maker, post-Basket Maker, pre-Pueblo and finally Pueblo. Among the Pueblos, the making of pottery reached a high state of development. Furthermore, styles in pottery changed from time to time so as to indicate a series of subdivisions of Pueblo cultures down to the arrival of the Spaniards (1640) and the present day. It may be noted that the skulls of the Basket Makers were generally long and those of the later Pueblos generally broad. These studies make it possible to give relative dates to many burials with a close approach to certainty (Kidder).

Through the brilliant researches of Douglass, and with the use of his "tree ring" method, it now appears possible to assign exact dates to some of the pueblos. It is stated that one timber at Cliff Palace, Mesa Verde, in Colorado, was cut in 1073 A.D.; others from Pueblo Bonito in Chaco Canyon, New Mexico, were cut in 919 and 1130 A.D. (Douglass, Judd).

The age of these prehistoric ruins seems to be not quite so great as has been supposed. Some of the most important ancient bones that in all reasonable probability are syphilitic come from the Pueblo area.

In Mexico and Central America one encounters the only parts of the American continent where a native written language developed. Through the excavations and studies of recent years much progress has been made in deciphering the Central American hieroglyphics, particularly those of the calendar. A basis for dating relics is, or soon will be, available. Up to this time, little skeletal material bearing on the subject of ancient syphilis has been secured from this region. It must be regarded as a most promising field for future work. However, many bodies were destroyed, owing to the practice of cremation.

Ancient Peru, for an American Indian community, had a large population. The methods of burial and the dry climate and soil have preserved for us an enormous number of skeletons and mummies. The origin and succession of cultures in Peru are matters about which there is much controversy (Kroeber, 1926 and 1930). However, it is usually possible to distinguish remains of the early periods from those of the Inca period. Tradition indicates that the Inca period began about 1200 A.D. Some of the most important evidence for ancient syphilis has been assigned by its discoverers to the pre-Inca period, and it would therefore be pre-Columbian.

Those who believe that historical evidence shows that syphilis originated in America claim that it was conveyed to Spain by sailors of Columbus, who became infected in the island of Haiti. One might expect evidence in the form of diseased bones to exist in Haiti or Cuba or the adjacent islands. There are several difficulties in the way of producing such evidence. Comparatively little archeologic exploration has been done in these islands. The conditions of burial and of climate are not favorable to the preservation of human remains. The

native Indians have been replaced, particularly in Haiti, by a population of Negroes or persons of mixed bloods. Yaws and probably syphilis are prevalent in Haiti. Any syphilitic bones discovered would need to be subjected to the closest scrutiny to determine their antiquity and to exclude the possibility of a recent burial. However, bones that are in all probability syphilitic and that are probably pre-Columbian have been uncovered in Florida (cases of C. B. Moore and Lamb, described hereafter), and Florida is near to Cuba and no great distance from Haiti.

#### THE PROBLEM OF DIAGNOSING SYPHILIS IN DRIED BONES

For the purposes of this investigation I have devoted considerable time to obtaining a rudimentary knowledge of archeology in the field, and to study of bones affected by syphilis, particularly skulls. I have examined such skulls in London, Paris, Amsterdam, Leyden, Berlin, Leipzig, Munich, Prague, Vienna, Zurich, Boston, Philadelphia and Washington, to mention only museums having fifteen or more skulls labeled syphilitic. I have notes on more than five hundred modern skulls with this diagnosis. The list of cities given is not without significance, for it enables one to say that there seems to be no difference of opinion as to what constitutes a typical syphilitic skull. Not all the skulls labeled syphilitic are perfectly typical; I should estimate that fewer than half of them are, but I should think the perfectly typical syphilitic skull, to be described hereafter, a more certain criterion for diagnosis than a Wassermann reaction of four plus or an aneurysm of the aorta. With long bones the case is different; however, with the assistance of the x-rays and of sections examined under the microscope, a high degree of probability in the diagnosis may be obtained with some long bones.

#### DESCRIPTION OF SYPHILIS OF BONE

Except in osteochondritis syphilitica of infants, the bones are usually involved late in the course of syphilis. Two principal forms of syphilis of bone are recognized; gummatous and diffuse, or nongummatous. As the gummatous form is more important in connection with the skull and the diffuse form in connection with the long bones, they will be discussed under those headings.<sup>5</sup>

*The Syphilitic Skull.*—The type of skull that is most characteristic of syphilis is that produced by widespread gummatous inflammation of the periosteum, frequently with localized gummatous nodules. The gummatous periostitis is accompanied by a certain amount of osteitis. It leads to destruction of bone and simultaneously or later there is new formation

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5. I have avoided the use of the adjectives specific and nonspecific in this connection, as they seem to me misleading. They are just as appropriate for any other infection as they are for syphilis.

of the bone at the edges of the parts affected. An unevenness or irregularity of the surface of the skull results that is peculiar and distinctive. English books sometimes speak of it as appearing "worm eaten." Erosions bordered by new bone may be present where there were gummas, partly healed. As Virchow stated it, the scars remaining after a gummatous peripheral osteitis are most characteristic; there is deficiency of new formation in the middle with excess at the outer parts. Such scars produce jagged, linear, not rarely stellate depressions, with the most marked defect in the middle, and with smooth, rounded and elevated edges. Virchow revived the term "*caries sicca*," used by an earlier author, Bertrandi, for this process; that is, caries not accompanied by the pus that goes with acute osteomyelitis and periostitis (fig. 1).

The alterations produced by syphilis frequently affect a large part of the outer surface of the calvaria; the inner surface is rarely involved. They often begin on the frontal bone, but not always. The skull may be abnormally heavy, but is not necessarily so. Gummas may erode so deeply as to cause perforation. Large sequestrums are sometimes formed. Such skulls seem to be less common in congenital than in acquired syphilis (M. B. Schmidt).

The characteristic syphilitic skull used to be found at autopsies in hospitals more frequently than it is now. The same is true of gummas of the viscera, which, all reports indicate, are much rarer than they were thirty or more years ago. This is probably the result of better diagnosis and treatment, though some observers believe that the virulence of the organism that causes syphilis has diminished, speaking only of syphilis as it is now seen in the white race.

Those syphilitic skulls that I have referred to as being not characteristic show various thickenings, protuberances, scars, defects and sometimes necrosis without healing. If there were no other evidence of syphilis present, a diagnosis would be impossible. Some of these cases undoubtedly result from the diffuse, nongummatous form of syphilitic periostitis. The latter produces thickening of the outer surface of the skull, which may be either rough or smooth, and which has no feature that I know of distinguishing it from thickening produced by other causes of chronic periostitis.

Late syphilis is well known to involve the nasal region or hard palate in a considerable proportion of cases. It usually begins as an ulceration of the mucosa that extends into the bone. As the nasal region is often involved in lupus, in yaws (*frambesia*) and in tropical leishmaniasis, particular care must be exercised in respect to ancient skulls in which the nasal region is the principal part involved. Cases of this kind will appear in some of the material to be discussed farther on.

The notched teeth of congenital syphilis (Hutchinson's teeth) might help establish the diagnosis of syphilis, though not by themselves entirely diagnostic.

In a personal communication, Sir Arthur Keith informed me that the Museum of the Royal College of Surgeons, London, had recently



Fig. 1.—Typical syphilis of the skull, of severe type—"caries sicca." The specimens are in the museum of the Rudolf Virchow Hospital (Charité), Berlin; the upper skull is 18802. The photograph for this paper was made by E. Engelen, Berlin, through the courtesy of Professor Lubarsch. (Original photograph.)

acquired from the Society Islands a skull with lesions resembling those of syphilis, but attributed to yaws; the diagnosis of yaws and the exclusion of syphilis would need to be established to a certainty if this

unusual specimen is to be accepted. Osteomyelitis with periostitis apparently does not produce alterations on the skull comparable to those seen in typical cases of syphilis, and I do not know of any such specimen. Tuberculosis may produce destruction of bone, but there is not usually any noteworthy formation of new bone. Professor Tendeloo, of Leyden, called my attention to a skull in his collection from a case of leontiasis ossea in which the highly irregular upper surface of the cranium considerably resembled that seen in healed gummatous periostitis, but the characteristic stellate scarring was lacking, and the enormous thickening of the skull, with the other features of the case, would make confusion most improbable. Several writers have described as syphilis in ancient skulls what appear to me to be examples of symmetrical osteoporosis of the cranium, a disease that was common in some ancient races (Williams, 1929). Lancereaux suggested that leprosy may have produced alterations on the surface of ancient skulls similar to those of syphilis; little information seems available on this aspect of the subject. Leprosy might need to be considered in the case of ancient bones from Europe, Asia and Africa; there is no evidence that it existed in America prior to the discovery by Columbus. Erosion by water and substances dissolved in it, by roots and by rodent animals should not lead to errors on the part of those accustomed to the appearance of ancient bones.

*Syphilitic Long Bones.*—The gummatous form may occur as in the case of the skull, with destruction and new growth of bone, healing and scar formation. It may affect the interior of the bone, but more often it occurs peripherally. The epiphyses and joints are occasionally involved. It has frequently been noticed that bones that are exposed to injury, such as the top of the skull, the tibia and the clavicle, are likely to be affected, injury determining the location of a gumma. So many conditions produce localized destruction of bone with subsequent thickening and formation of new bone that the diagnosis of this form of syphilis in a single dried bone without its clinical history would be dubious; the involvement of several bones would make a diagnosis of syphilis more plausible. In the case of long bones, few observers lay much stress on the stellate scars described by Virchow as characteristic of syphilis, although such scars are of great importance in the skull. Large areas of necrosis, leading to the formation of sequestrums, may occur; they may be due to secondary infection. Such sequestrums are less frequent in syphilis than in osteomyelitis and tuberculosis. In sections of fresh preparations, the histologic picture of gummatous granulation tissue is present (fig. 6A). Spirochetes are said to be rarely demonstrable.



The diffuse, nongummatous form of syphilis of bone has been described especially by German writers. It is often encountered in long bones, and several bones are likely to be involved. This form of syphilis, or something much like it, is largely responsible for the difficulties in diagnosis encountered in ancient dried long bones.

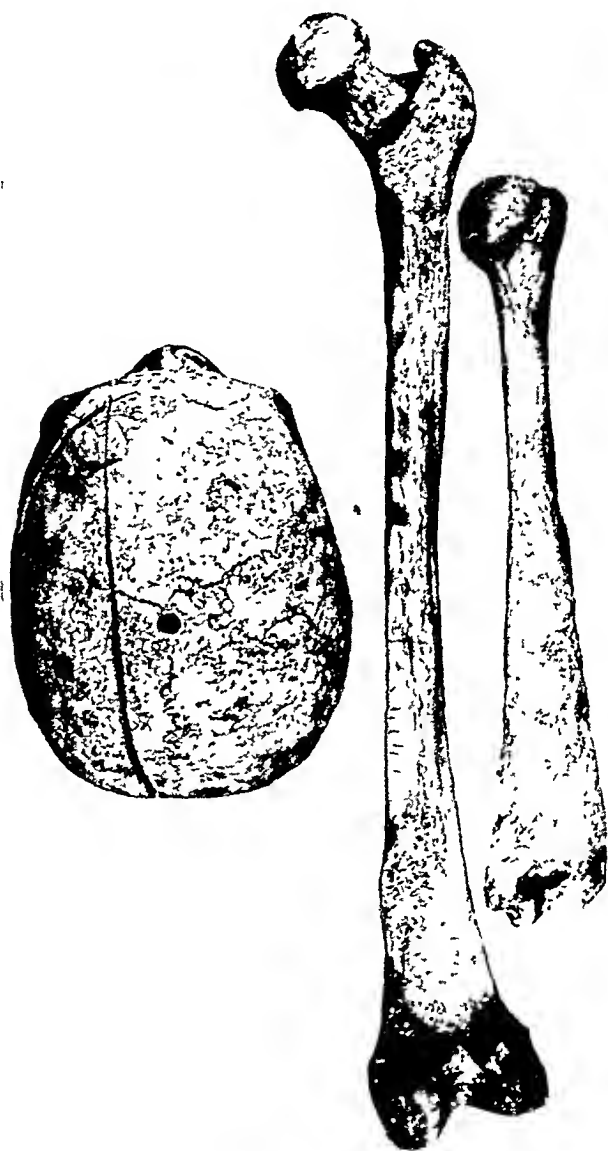


Fig. 2.—The skull and bones of a prostitute, Army Medical Museum, Washington, D. C., no. 6414. The photograph and roentgenogram were furnished by Major George W. Callender. The long bones appear to show both gummatous and diffuse syphilis of bone.

In fresh specimens of this diffuse type an osteoperiostitis is found the histologic appearances of which are said to have nothing characteristic of syphilis. However, the gummatous and the diffuse form may occur

in one and the same bone (figs. 2 and 3). Some observers attribute this periostitis to toxins, but in view of the uncertainty in regard to the pathogenesis of other late syphilitic lesions it is safest to maintain an attitude of reserve in respect to the diffuse periostitis, although the term is useful in classification.



Fig. 3.—A roentgenogram of the long bones shown in figure 2.

The diffuse form of bone syphilis affects the shapes of long bones. Enlargement occurs from new growth of bone beneath the periosteum. The outer surface may be nearly smooth or rough, sometimes extremely rough, suggesting a mixture of the gummatous with the diffuse type. There may be considerable osteophytic growth. The areas of thickening with smooth surfaces may show many small openings for the trans-

mission of blood vessels and often longitudinal grooves. The anterior border of the tibia is a frequent location for such new growths, and a saber-shaped tibia may result, but this is no longer regarded as distinctive of syphilis. Osteitis may occur along with the periostitis, producing new bone that leads to narrowing of the medullary canal;

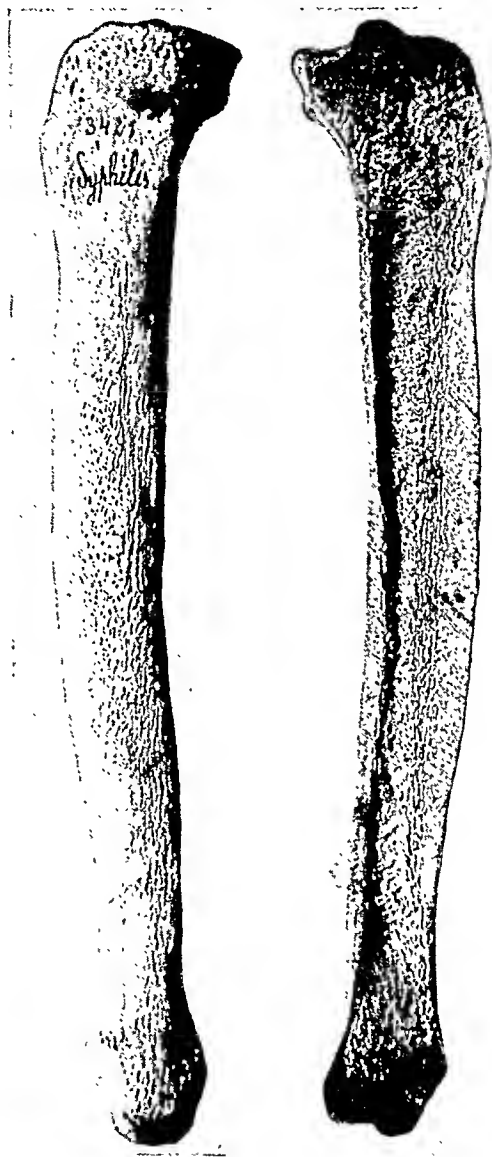


Fig. 4.—Syphilis of the tibia. The specimens are in the Pathological Institute, German University, Prague. The photograph was furnished by Prof. A. Ghon. Note the openings for blood vessels, many of them running longitudinally on the outer surface. On the right hand side of the section of the bone, the so-called "border stripe" is visible in the photograph, though not very distinct; this indicates a new growth from the periosteum.

such new bone may be dense like ivory; it is heavier than normal (figs. 4 and 5).

In general, investigators lay stress on the new formation of periosteal bone in syphilis (fig. 6). In dried bones, such periosteal growth may be demonstrated by means of the x-ray or in sections, gross or microscopic. The use of the x-ray for the diagnosis of syphilis of bone has been developed especially by Ludwig Pick and his pupils. According to Wilhelm, roentgenograms show marked formation of periosteal

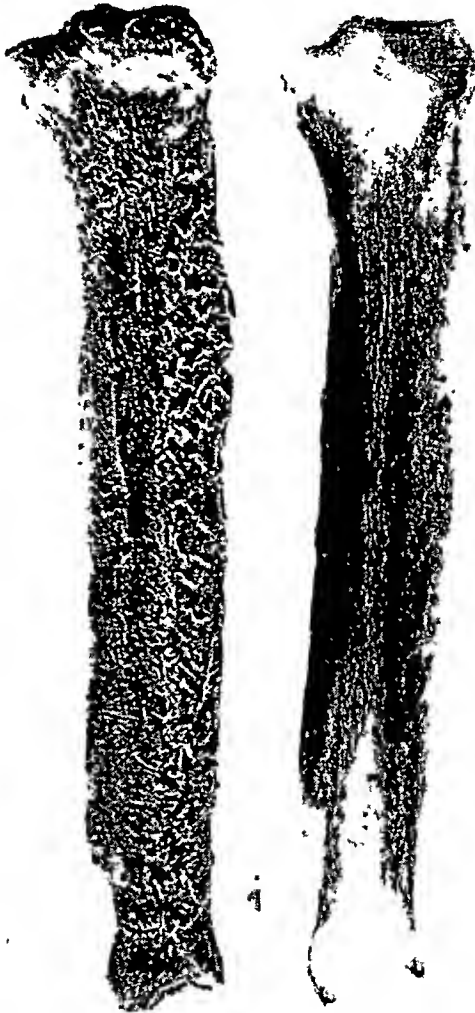


Fig. 5.—Syphilitic osteoperiostitis. The photograph and roentgenogram are published by the courtesy of Prof. Ludwig Pick, Berlin, and Dr. Seymour Wilhelm, New York. This is case 4 of Dr. Wilhelm.

bone, more or less narrowing of the marrow cavity and a sclerotic or finely porous quality of the new bone tissue; such fine meshes run in the long axis (fig 5). Articles by Weber, by Nestmann and by Michäelis have appeared recently, all having for their objective the diagnosis of syphilis in ancient bones by means of the microscope. Weber laid stress on the detection of a stripe (*Grenzstreifen*) marking

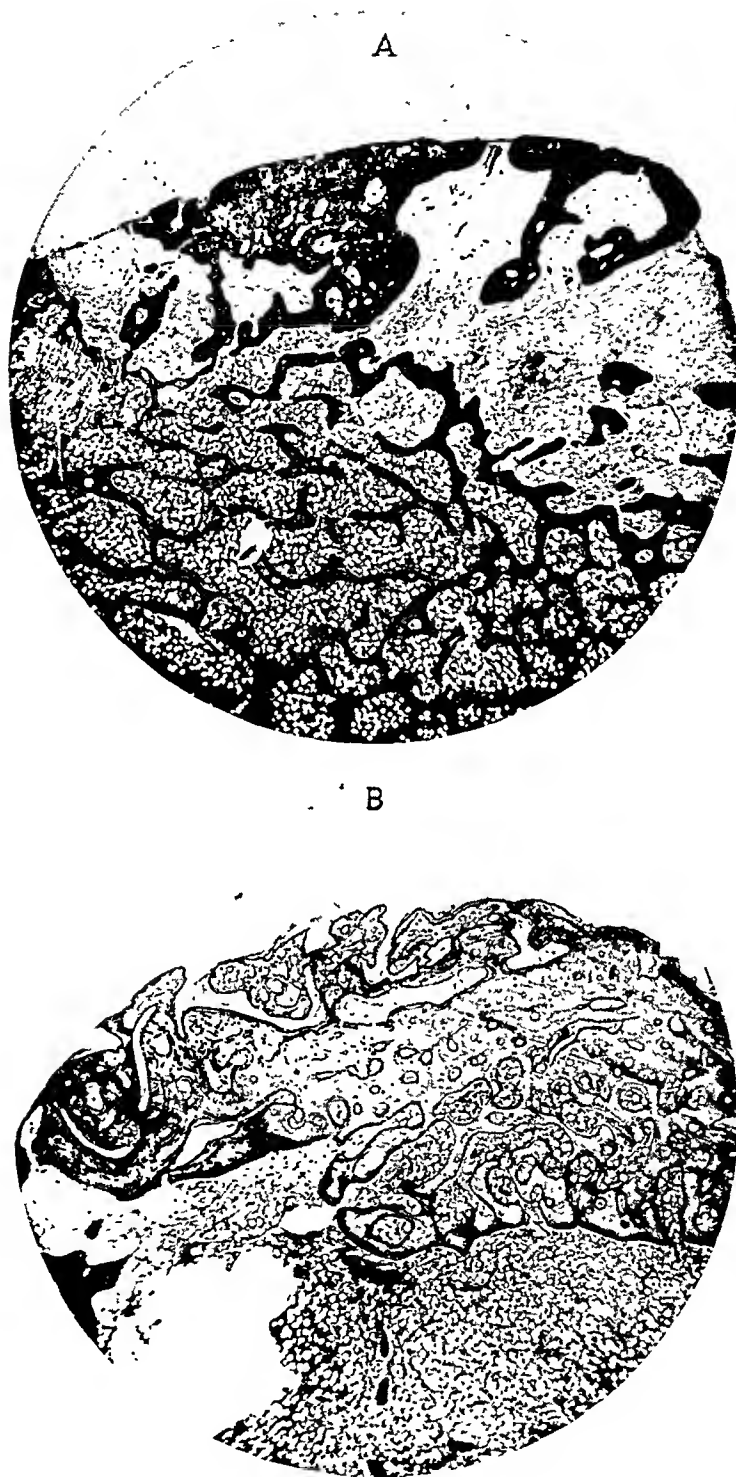


Fig. 6.—Syphilis of a long bone;  $\times 10$ : *A*, active gumma; *B*, new formation of bone from the periosteum, a short distance from a gummatous lesion. Several long bones were involved. (Case from the Buffalo City Hospital.) (Original photograph.)

the boundary, where the new periosteal bone has been laid down on the old bone. He emphasized the value of detecting Sharpey's fibers in the new periosteal bone; in my experience, Sharpey's fibers are so difficult to see that they give aid only occasionally. In cases of long standing, the new periosteal bone may show haversian systems with concentric lamellae; the transition to the underlying bone may be gradual, and the boundary stripe not discernible. Michäelis thought that Wilhelm and Weber had overestimated the importance of periosteal growth of bone in syphilis; one may be unable to demonstrate it in cases of undoubted syphilis.



Fig 7.—Periostitis and osteomyelitis with new growth of periosteal bone;  $\times 10$ . The case (University of Buffalo case 6182) was one of compound fracture of the femur in a child, with resulting infection. No callus formed. The femur was amputated. A mantle of new, soft, periosteal bone extended along the shaft of the femur for 15 cm from the seat of fracture. The marrow spaces were crowded with leukocytes. (Original photograph.)

#### DIFFERENTIATION OF SYPHILIS FROM OTHER PATHOLOGIC CONDITIONS

Acute osteomyelitis with periostitis may also lead to new growth of periosteal bone (fig. 7), though less frequently than does syphilis. It is more likely, than syphilis, to affect single bones and to lead to the formation of sinuses and sequestrums. The appearances of the bones vary enormously in different cases. In the absence of other data, in a single

dried bone it may be quite impossible to distinguish it from syphilis by any means of diagnosis known. Tuberculosis commonly causes destruction, but not regeneration, of bone. In a considerable number of rather rare chronic infections, such as leprosy and actinomycosis, the bones, it is said, may be affected, and some new growth may occur; on account of their rarity, these are not likely to lead to errors in diagnosis. Yaws (frambesia) is said to show tertiary lesions of bone similar to those found in syphilis and giving a roentgenogram like that of syphilis. Some roentgenograms from cases of tropical frambesia sent me by Professor Schüffner, of Amsterdam, show proliferation of bone different from any I have seen in syphilis or other conditions. The bone lesions of yaws seem not to have been thoroughly studied; from the information available I should think they were much less frequent and less extensive than those of syphilis.

In view of the tremendous new growth of bone often seen in the healing of fractures it cannot be doubted that new growth of bone takes place after injuries of the periosteum in which there has been no visible fracture and no infection.

The rather rare hypertrophic pulmonary osteo-arthritis of Pierre-Marie (Crump) may lead to general formation of osteophytic growths that might be mistaken for those seen in syphilis. Sjövall and Michäelis believed that this mistake in diagnosis was made in the case of the skeleton of Magnus Ladulös, referred to farther on in this article.

In rickets there is sometimes new growth of periosteal bone; however, rickets has rarely been found in bones from ancient times. Symmetrical osteoporosis of the cranium may show osteophytic growths; it was common in ancient America, but should be recognized by its gross appearance (Williams, 1929).

The condition, or conditions, called osteitis deformans, Paget's disease, osteitis fibrosa (of von Recklinghausen) or osteodystrophia fibrosa may produce changes in the bones indistinguishable to the naked eye from the diffuse, nongummatous form of syphilitic osteoperiostitis. This subject was discussed at great length at a meeting of the German Pathological Society in 1926 (Christeller). At that time the tendency among many German pathologists seems to have been to consider the osteitis fibrosa of von Recklinghausen and the osteitis deformans of Paget as different phases or different stages of one and the same process. That is the attitude of Kaufmann, in the American edition of his "*Lehrbuch der pathologischen Anatomie*," translated by Reimann, 1929. In some more recent articles, and since this portion of the present paper was first written, certain observers, who formerly regarded osteitis fibrosa and osteitis deformans as aspects of a single process under the

name of osteodystrophia fibrosa, now consider them entirely different processes. Among English pathologists, Turnbull, in a publication of October, 1931, discussed their points of resemblance and difference without stating a definite opinion. Lang regards osteitis fibrosa as a secondary result of some preexisting disease of the bones; he believes osteomalacia and rickets are the most important underlying factors in

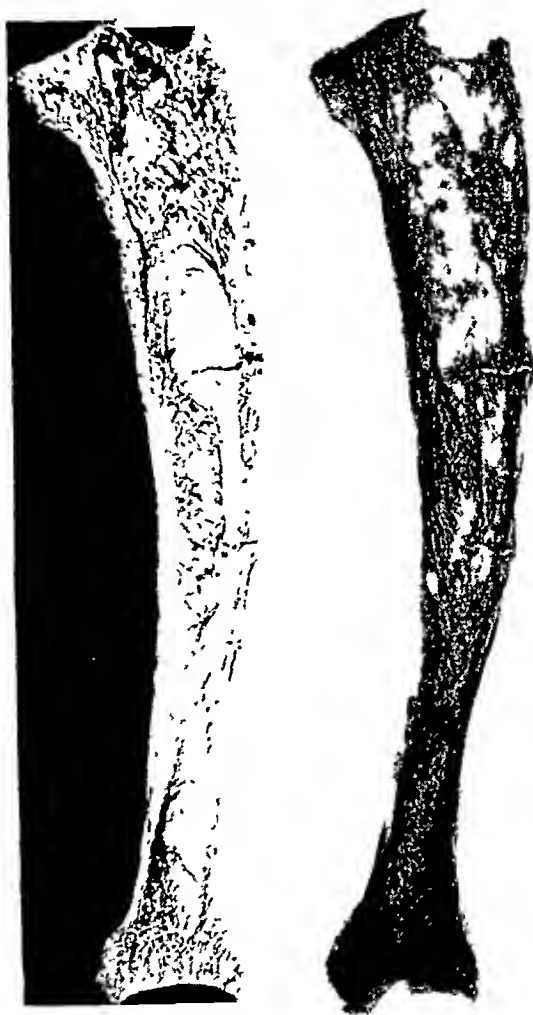


Fig. 8.—Osteodystrophia fibrosa—Paget's disease—a saber-shaped tibia of a man, aged 77, with arteriosclerosis. The photograph and the roentgenogram are published by the courtesy of Prof. Ludwig Pick, Berlin.

cases of widespread osteitis fibrosa. Knowledge of the relations of the diseases in this group to one another is passing through a transitional period at present. One is sometimes uncertain as to an author's meaning when he writes of osteitis fibrosa or osteitis deformans. Furthermore, some cases are difficult to classify on morphologic evidence alone (see figs. 9 and 10).





Fig. 9.—Osteodystrophia fibrosa, active, still progressing (case 6131, University of Buffalo). The roentgenogram permits one to see the phalanx in the center without much reduction in size. The roentgenogram was furnished by the Marine Hospital, Buffalo, N. Y. The classification of this case is somewhat in doubt; some features of it even suggest osteomalacia: It occurred in a man, aged 56. There were two fractures of the right femur and one of the right tibia; the left femur was bent outward in its upper part. Roentgenograms showed slight thickening and some mottling of the vault of the cranium. Several of the bones of the arms showed lamination, and one humerus and ulna presented definite thickening from periosteal bone. The pelvis was mottled with light and dark areas. The right femur and tibia showed lamination. When the patient was last seen the x-ray pictures had revealed no tumors and no cysts; no examination for parathyroid tumors had been made. The involvement of the bones of the hand is said to be unusual. Figure 10 is a photomicrograph from the right femur of this patient.

Schmorl's recent article is based on the study of fifteen cases of generalized osteitis fibrosa and one hundred and ten cases of osteitis deformans. It seems to me that these diseases must be commoner in Europe than in the United States. I have seen only a few cases of them. Schmorl found cysts of bone, "brown tumors" (presumably giant cell tumors) in the bones, and the (so-called) tumors of the parathyroid bodies in his cases of osteitis fibrosa; he did not find them in his cases of osteitis deformans of Paget. Although a final verdict on the pathogenesis of these diseases must be deferred, it seems that, while the microscopic structures of the two are very similar in the early

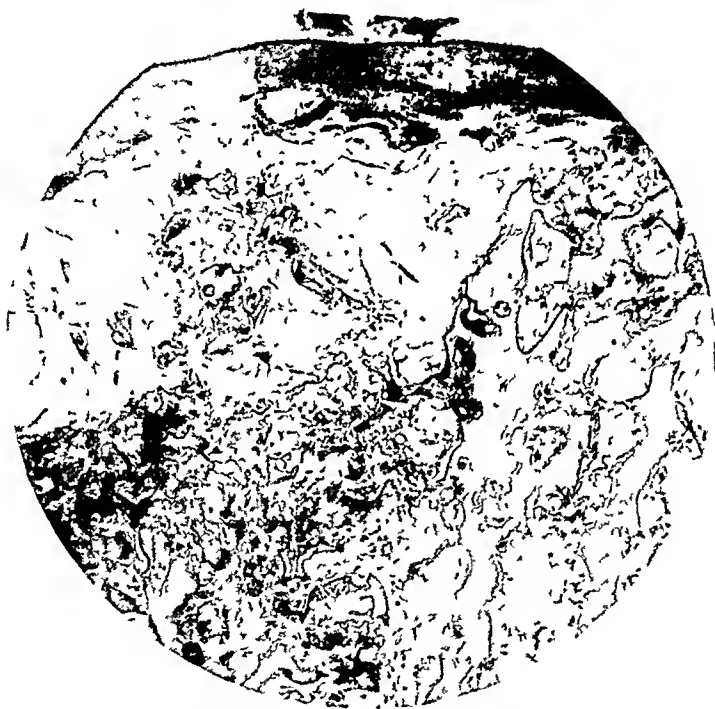


Fig. 10—Osteodystrophia fibrosa (case 6131, University of Buffalo); right femur; section stained with hematoxylin and eosin;  $\times 14$ . The section shows the fibrous and cellular marrow, with some fatty marrow, and a small amount of irregularly disposed new formed bone. High power magnification shows many osteoclasts and osteoblasts, and poorly developed mosaic structures in the new bone. See figure 9 for a roentgenogram from the same case. (Original photograph)

stages, the amount of new soft bone formed is greater in Paget's osteitis deformans. This is, therefore, the form more likely to be confused with syphilis in making a diagnosis on dried bones. Denninger recently described osteitis fibrosa in the skeleton of a prehistoric American Indian; Pales reported cases of osteitis deformans and osteitis fibrosa in neolithic bones from France.

Paget's osteitis deformans is likely to involve a large number of bones in the same person, although single bones may be affected. The bones are enlarged, their surface may be irregular, they may bend, there may be a saber-shaped tibia, the marrow cavity is usually large or is filled with spongy bone. Fractures of bones may occur, but they are less frequent than in osteitis fibrosa, according to some observers. The fully formed new bone is said to look, on section, like pumice stone. The bones sometimes present cavities like cysts (Turnbull); cysts are said to be rare. The skull may be very thick. Some investigators regard certain cases of leontiasis ossea as belonging in this cate-



Fig. 11.—Mosaic structures from a section of the skull in a case of Paget's disease (not the case shown in figure 8). The photograph was furnished by Prof. Ludwig Pick.

gory. It can usually be shown that the changes in Paget's disease do not lead to new formation of bone by the periosteum as is so commonly the case in syphilis (see Freund and Schmorl in a later paragraph).

According to Ludwig Pick and his pupils (Wilhelm), the roentgenogram gives a characteristic picture in Paget's osteitis deformans: The marrow cavity is large, the new formed bone appears split into lamellae running lengthwise with the bone, and most characteristic of all is the absence of any new growth of bone from the periosteum (figs. 8 and 9). However, "compensatory" periosteal growth apparently may occur in rare cases of Paget's osteitis deformans (Weber). I have a roentgenogram that I interpret as being from such a case. Freund attributed the thickening of the shaft of long bones to "parosteal" growth in the

adjacent fat and connective tissue. Schmorl (1930) found new periosteal growths of bone as much as from 5 to 6 mm. in thickness; the mosaic structures described in a subsequent paragraph were lacking in them. Pick thought that such growths, occurring coincidentally with growth in the cortex of the bone, would not appear in roentgenograms as being separated from the cortical growth.

Most observers agree that the changes in Paget's osteitis deformans are not inflammatory. Recent studies suggest that tumors or hyperplasias of the parathyroid bodies or excessive secretion from these bodies of a hormone influencing calcium metabolism may be the cause in some cases of osteitis fibrosa (Hunter and Turnbull; Jaffe, Bodansky and Blair). There is evidence suggesting that some cases called osteodystrophia fibrosa or osteitis fibrosa (Christeller, Grauer) result from improper food; that may have been important among ancient and primitive people.

Whatever the cause may be, the process consists of a resorption of bone with rebuilding of irregularly disposed bone (fig. 10). The formation of new irregularly disposed lamellar bone produces in its final stages what Schmorl called "mosaic structures." Sections of the new bone, under moderately high power, show that it is traversed by numerous short, irregularly running lines of cement substance, giving the impression that the tissue is made of many small pieces put together like a mosaic (fig. 11). Schmorl regarded the mosaic structures as pathognomonic of osteitis fibrosa in his paper of 1926. More recently (1930), he separates osteitis deformans from osteitis fibrosa, where he formerly regarded them as aspects of one process. He now finds the mosaic structures extensively and typically developed in osteitis deformans, while they are fewer and more regular in their course in osteitis fibrosa. An illustration in Paget's original article seems to me to show the mosaic structures. Freund remarked that the cement lines are broad, and that they stain deeply. The mosaic structures are well illustrated in Schmorl's article of 1930.

As the mosaic structures could be demonstrated in dried specimens, Schmorl suggested using them to differentiate between osteitis fibrosa (I understand that he would now say osteitis deformans) and syphilis in ancient bones. Nestmann found that the mosaic structures were present over limited areas in one case of syphilis of bone, his observation being verified by Schmorl. Michäelis was apparently not aware of Nestmann's observation when he said: "The mosaic structures separate osteodystrophia fibrosa from all forms of bone syphilis." Pick has seen mosaic structures in recent syphilitic hyperostoses. Schmorl said that they appeared in other bone diseases also, but that these were not comparable to the mosaic structures of osteitis deformans.

## TECHNICAL POINTS IN EXAMINATION OF ANCIENT DRIED BONES

In my experience, careful examination with the naked eye, including inspection of the bone after it has been sawed lengthwise, furnishes more information than can be obtained by any other single method. By these means one can usually determine whether or not a new growth of bone is periosteal in origin. The dried bones should be moistened slightly; they can be sawed better when they have been soaked for several hours in about 5 per cent formaldehyde solution. Weber insisted that in preparing sections for examination with the microscope, pieces be taken from several parts of the bone at some distance from one another. Even in old bones preliminary fixation in formaldehyde is desirable. Decalcification in 1 to 5 per cent nitric acid and embedding in celloidin may be carried on as described in any book on histologic technic. With fragile material, decalcification with nitric acid may be accomplished after embedding in celloidin. I have found that, except for the cells, the minute structure may be preserved perfectly in very ancient bones from countries having a dry climate, such as Egypt and Peru. More recent bones, presumably buried in a moist soil, as from the southern part of the United States, have lost a large part of their organic matter, and sometimes are infiltrated with mineral salts, presumably calcium; sections can only be made by grinding. The use of the expression "fossil bone" to indicate that a specimen is ancient, as is often done by anthropologists, is misleading; recent bones may be fossilized and old ones not. Microscopic fields having the original framework well preserved may lie haphazard among fields where the structure is blurred or lost.

Weber experimented with ground sections of bone, both thin, for transmitted light, and thick, which he examined by reflected and polarized light; the use of thick sections he considered applicable to ancient bones in which the organic matter had been replaced by calcium and other salts (so-called fossil bones). His technic was admirable and was worked out with immense detail, for which his article must be consulted. He found that with thick sections examined by reflected light he could demonstrate enough of the finer structure of bone to be of assistance in differentiating syphilis from other diseases of bone, such as Paget's osteitis deformans. Michaelis applied the technic of Weber to recent normal and diseased bones; also to some ancient bones in the case of which the diagnosis was in doubt. His results will be considered on the succeeding pages. Both Weber and Michaelis gave good photomicrographs of their sections. In some of their plates new growth of periosteal bone is evident, but I must admit that I am unable to see some of the finer histologic changes that they describe.

## SUMMARY

The skull is most important: Extensive involvement of its upper surface, the so-called "caries sicca," new formation of bone, irregular and stellate scars and the "worm eaten" appearance justify the diagnosis of syphilis in all reasonable probability. Involvement of the nasal region, with new growth of bone is frequent, but caution must be used in these cases, as there are several other infections that may affect the nasal region, usually without much formation of bone, however.

In syphilis, in nonsyphilitic periostitis and in Paget's osteitis deformans, the shafts of the long bones may show irregular thickenings. It is usually impossible to differentiate between these conditions by examination of the exterior of a given long bone. The bone should

be roentgenographed, sawed lengthwise and sections made for examination with the microscope. In some cases, the diagnosis must remain in doubt.

In syphilis, the new-formed bone is largely periosteal in origin and often dense; encroachment on the medullary canal is characteristic. Sinuses and sequestrums are uncommon. Involvement of two or more bones of the extremities is in favor of this diagnosis.

In nonsyphilitic periostitis, the new-formed bone is usually periosteal. It is unlikely to encroach on the medullary canal. In periostitis connected with infectious osteomyelitis, the bones are likely to have sinuses and sequestrums. Usually single bones are involved.

In Paget's osteitis deformans, the new-formed bone is rarely periosteal, but future studies may show that periosteal bone is formed more often than has been believed. The new bone is usually not dense, but porous. The medullary canal is likely to be large. Usually several bones are involved. The mosaic structures described by Schmorl may eventually be of assistance in making the diagnosis; I have not found the results described to date convincing.

The recognition of *Spirochaeta pallida* in doubtful ancient bones, a suggestion that has come to me from some anthropologists, hardly merits serious consideration. It is just possible that spirochetes could be detected in the dried soft parts of mummified bodies. The recognition of syphilitic aortitis in a mummy is well within the range of possibilities. I have been able to secure a good specific stain in the elastic tissue of the aorta of an Egyptian mummy of the Eighteenth Dynasty (about 1000 B.C.).

#### REVIEW OF ALL REPORTED CASES OF ANCIENT SYPHILIS OF BONE

It is probable that I have overlooked some reports, as many of those that I have found were published in journals of archeology and ethnology, or in the transactions of similar societies and museums. Such publications are often difficult of access, and apparently no general index exists for all of them. I hope that nothing important has been overlooked. Considerable confusion has been created when a case or a specimen has been described by different authors under different names. Although many specimens have been described as showing ancient syphilis, in only a few of the cases are the presentations plausible enough to make them important. Those that seem to me not important I am considering as briefly as possible, but I am including them for the sake of completeness. The important cases are described in greater detail and are discussed in the summary at the end of this paper. For the sake of convenience the cases will be taken up by countries beginning with Japan.

## JAPAN

Adachi described certain long bones from shell heaps in Japan, stated to be of the stone age, i. e., more than twenty-five hundred years old, that show changes which were pronounced by Yamagiwa to be those of syphilis. The same specimens were described by Dohi, with illustrations; Dohi did not feel certain that they are syphilitic, and was also uncertain as to their antiquity.

These bones are a tibia and a fibula, both showing thickening and roughening of the shaft; the two are united by a kind of bridge of bone about their middle. The outer surface of the tibia presents a depression that might have been produced by ulceration. Dohi noted that the stellate scars described by Virchow as characteristic of syphilis are not present. I have not been able to secure original photographs of these bones. The illustrations published by Dohi are not clear enough to warrant reproduction. His illustration showing the bridge of bone referred to seems to me to suggest injury as a cause for the changes seen, or possibly a healed osteomyelitis with periostitis. I do not know of any roentgen or microscopic studies made of these bones.

## EGYPT

Certain bones from Egypt, said to be more than twenty-eight centuries old, were described in 1900 by Dr. Zambaco, pasha, as syphilitic. I am indebted to the article of Raymond (1911) for the facts. It was stated that Fournier did not agree with Zambaco, and that the matter was referred to a committee of the Academy of Medicine of Paris, but the committee never rendered a report. Raymond seems to have rejected these specimens. They were also referred to by Bloch, who quoted the criticisms of Bayet and Fournier.

*The Roda Skull.*—This skull has been described by Lortet and by Lortet and Gaillard. It is now in the Museum of Natural Sciences, Lyons, France, where I had the opportunity of examining it carefully in August, 1925 (fig. 12).

The skull is stated to have come from a prehistoric cemetery at Roda, Upper Egypt. It is a small skull, probably belonging to a young woman. The lower jaw is missing; the last molars in the upper jaws are not erupted; the teeth have fallen out post mortem, except three bicuspid, which are in good condition. Part of the left temporal bone has been broken off post mortem. The palate and facial bones are normal. The skull is remarkably well preserved for one of such antiquity; it has a beautiful yellow-brown patina, like old ivory. The supposed syphilitic lesion consists in erosion starting from the outer table of the cranium. This erosion is less marked on the frontal, the right parietal and the occipital bone. It is most marked on the left parietal bone at its upper, anterior portion, where an irregularly shaped portion of the outer table has been removed, and at two points there are small perforations completely through the inner table. At several places, the patination is darker on the outer surface and becomes lighter according to the depth of the excavation. This, I should suppose, is what would have happened if the erosion had occurred after time had given the skull its brown color. There is no new formation of bone. In my opinion, this is not a syphilitic skull, and the erosion that it presents was the work of some rodent animal. Elliot Smith (1908) wrote of a

beetle that erodes bones in Egypt, and ascribed the changes in the Roda skull to this beetle, as well as the changes early described by Fouquet in bones from Egypt, regarded by Fouquet as evidences of syphilis. Gangolphe and Raymond also expressed the opinion that the Roda skull was not syphilitic. Apparently this is the skull referred to by Moodie in his "Paleopathology," p 411; the drawing that he gives does not look as though it could have been made from the same skull (Moodie's plate LXXXVI). I have introduced a photograph of this skull, as it affords an example of the kind of case that has led to confusion through incorrect diagnosis.

*Nubian Bones Described by Michaelis.*—Michaëlis described briefly certain bones furnished by Sir Arthur Keith from the Hunter Collection of the Royal College of Surgeons, London. These bones, dated



Fig. 12.—The Roda (Egypt) predynastic skull, the lesions in which, in my opinion, have been incorrectly diagnosed as syphilitic. According to Prof. G. Elliot Smith, the erosions were probably produced post mortem by beetles. The photograph was furnished by Prof. M. C. Gaillard of the Museum of Natural Sciences, Lyons, France, where the specimen now is.

around 1000 B.C., are said to be of Nubian origin; no other facts as to their origin are given. Of these specimens, a femur and a tibia are pronounced by Michaelis as probably syphilitic. From his illustrations of the gross appearance of these bones I should think his suspicion justified, although the pictures are too small and too indistinct to permit more than a guess, and other causes of periostitis would be equally probable. I can make nothing of his photomicrographs of the finer structure of the bones (Michaëlis, pp. 54-65 and pp. 85-87, especially p. 87. and plate XXXVI).



*Negative Evidence from Egypt.*—There is a large body of negative evidence as to ancient syphilis in Egypt. It is important because of the great antiquity of civilization in Egypt, with its attendant factors, the formation of large settled communities and the having commerce and intercourse with foreign peoples. Investigators of recent years, Elliot Smith, Wood-Jones, Derry, Dawson, Ruffer and Oettekling, are unanimous in stating that they have found not a single specimen of ancient bone from Egypt showing changes that could be called the result of syphilis. The fact that they usually made such statements is proof that they were on the lookout for this disease. Elliot Smith informed me that he had found not a single syphilitic skull in more than 25,000 ancient Egyptian skulls examined by him.

Ruffer and Rietti referred to unfinished excavations at Alexandria, Egypt, that would, when completed, include the crypts containing the skeletons of the prostitutes who accompanied the Greek army of Alexander the Great. The inscriptions on the tombs were said to give this information. They remarked, "Here, if anywhere, evidences of syphilis and gonorrhea should be found, provided venereal disease existed at that period." The excavations were in charge of Dr. Evaristo Breccia, curator of the Musée Greco-Romain at Alexandria. Dr. Breccia informed me that but two of these tombs were in question; these tombs have since been opened, but they had been invaded by mud and water, so that no useful information could be derived from their contents.

#### FRANCE

A considerable number of ancient bones from France alleged to show the lesions of syphilis have been described. Raymond discussed many of these cases, and I am indebted to his articles for a large part of the statements given in what follows, though I cannot agree with his conclusions. Some of these bones appear to have been mentioned first in a thesis by LeBaron (1881), which is not accessible to me; his most important specimen is said to have been lost. A certain amount of confusion has been created by the views of Parrot on a relation between congenital syphilis and rickets, views that have long since been discredited, but that some writers have taken seriously in recent years. Confusion has also resulted when various authors have discussed some of the same cases in different orders and under different designations (Buret, LeDouble, Gangolphe). Bloch referred to most of those described up to the time his book was written, 1911. A nearly complete list of them was recently made by Pales, who took a conservative position. Valuable testimony was given by Jeanselme, who said that the anthropologic collections of Europe contain no document not subject to criticism, and no bone or tooth that is a proof of the antiquity of syph-

ilis; he had made extensive researches in the museums of Paris, without result.<sup>6</sup>

I shall take up what seem to me the most important specimens first.

*The Tibia from Solutré, France.*—As far as I know, this specimen has not been the subject of any special article, although it is mentioned by several writers. In one of his two papers on the Roda (Egypt) skull, Lortet said that syphilis had been demonstrated in a female skeleton from Solutré, which had been carefully examined by Broca, Virchow, Parrot and Rollet, and that these investigators agreed that the changes shown on the tibiae were certainly syphilitic.<sup>7</sup>

In August, 1925, I examined this specimen, which is in the Museum of Natural Sciences in Lyons, to which it was presented by the finder, M. l'Abbé Ducrost (fig. 13). According to my notes, the right tibia had three enlargements anteriorly, one near the middle, one a little above it and another a little below it. The two lower were spindle-shaped and involved the anterior crest; the upper one was on the inner side. Each was about 4 or 5 cm. in length. The surfaces were smooth, except for being rendered a little porous by openings for the transmission of blood vessels. The bone was intact and had not been sawed at that time.

In my opinion, it is possible that these hyperostoses were caused by syphilis, but there is nothing characteristic about them, and there were several other possible causes besides syphilis, such as injury. The information that I was able to obtain indicated that the skeleton was regarded as prehistoric, but that no definite date could be assigned for it. In this connection, it may be pointed out that the dating of skeletal material from Solutré is beset with many uncertainties. The opinion credited by Lortet to Virchow is inconsistent with Virchow's own published utterances. Gangolphe and Raymond appeared to regard the Solutré tibia as giving evidence of syphilis, but agreed that its age is uncertain, though the specimen is ancient. Raymond stated that it may not antedate the barbarian invasion, and that it most probably corresponds with the Roman period. Several writers referred to this specimen without giving any new information. Pales recently reviewed the case and gave the most complete account of it that I have seen. He reproduced a roentgenogram of the bones that seems to indicate that the hyperostoses consist of rather dense bone. He also mentioned a small hyperostosis on the left tibia of the same skeleton. He seemed to think a positive diagnosis could not be made.

*The Long Bones of de Baye from the Valley of the Marne.*—These specimens have given rise to more discussion and controversy than any other find that I know of.

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6. "Les galeries anthropologiques d'Europe ne contiennent pas un seul document qui ne prête à la critique, pas un seul os, pas un seul dent, qui puisse être considéré comme une preuve de l'antiquité de la syphilis. Les longues et patientes recherches que j'ai poursuivies ici-même au Musée de l'Ecole et de la Société de l'Anthropologie ont été sans résultat."

7. ". . . on en (syphilis) a constaté la présence dans la station de Solutré, sur un squelette de femme, actuellement au Muséum de Lyon, et jadis examiné avec beaucoup de soins par Broca, Virchow, Parrot et Rollet, qui ont été unanimes à conclure que les alterations que présentent les tibiae de ce squelette sont certainement de nature syphilitique."

Certain burial caves artificially cut in the chalk of the valley of Petit-Morin in the region of the Marne, France, were explored by Baron J. de Baye. The results were reported at various times between 1874 and 1888. The collections made by de Baye were given to the museum at Saint-Germain. A study of the bones of this collection was undertaken by Raymond (1911, 1912), who found among them a humerus and an ulna that he believed showed evidences of syphilis.



Fig. 13.—Ancient tibia from Solutré, France. The nodes may have been produced by syphilis or may be due to some other cause. The photograph was furnished by Prof. M. C. Gaillard of the Museum of Natural Sciences, Lyons, France, where the specimen now is.

My information has been obtained from Raymond's article chiefly. Raymond stated that there could be no doubt as to the period to which the bones belong, that it was the latter part of the neolithic; a few bronze ornaments were discovered, indicating that the neolithic period was approaching its end.

The humerus and ulna in question were not known to have belonged to the same individual (fig. 14). They were carefully described as to

their gross appearance by Gangolphe. The humerus was heavy; it presented a hyperostosis over an extent of from 16 to 17 cm. of its lower and middle thirds, with small openings for the passage of blood vessels; the medullary canal was large; the thickening seemed to be due to the formation of layers of periosteal bone. The process was, how-

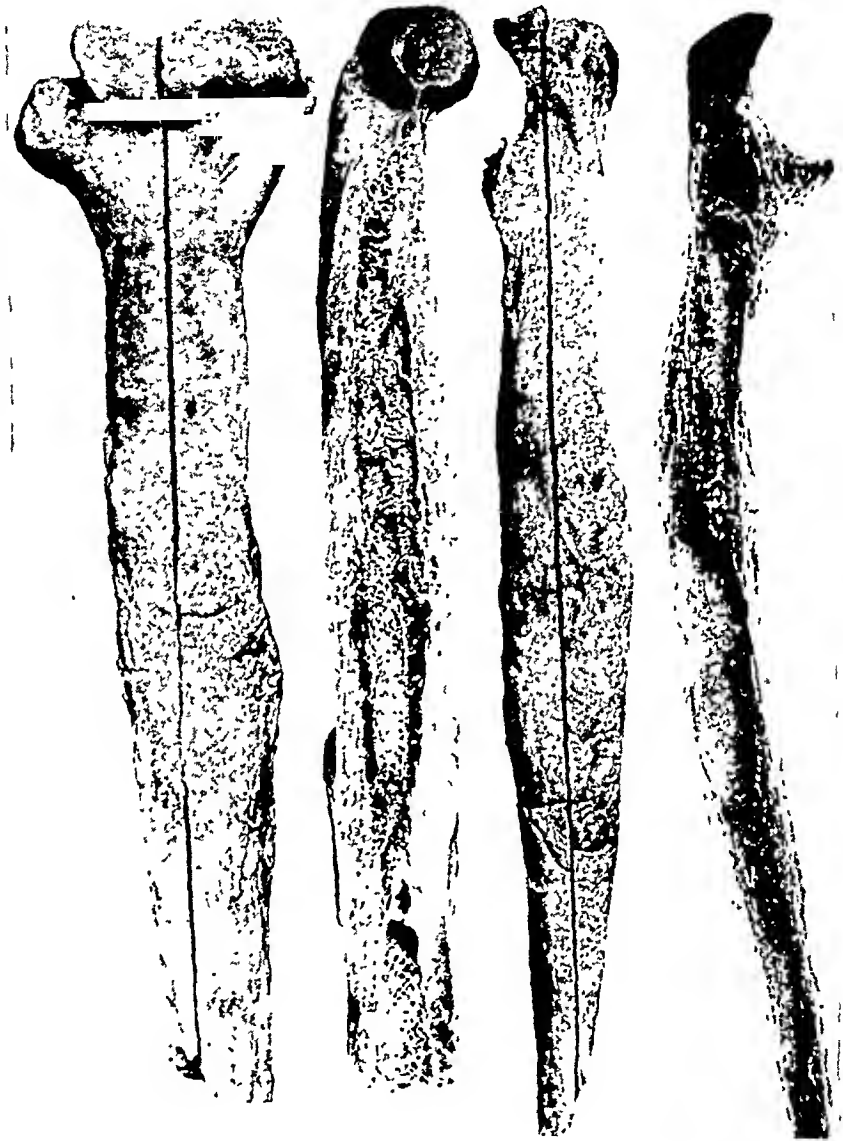


Fig. 14.—A humerus and an ulna found by de Baye in a burial cave in the valley of the Marne, France. They are said to be neolithic, and are regarded by some as showing the effect of syphilis. The illustration is copied by permission of Dr. Paul Raymond, Paris. Compare with figure 8.

ever, pronounced to be central and to have been a tertiary, gummatous, infectious osteomyelitis. The ulna was light; it was enlarged in the upper part of the diaphysis for from 13 to 15 cm., the surface showing

minute orifices. The new periosteal layers were traversed by fine canals, as were the remains of the old shaft; the medullary cavity was much enlarged, and in it was a cavity with definite walls. The ulna was regarded as having diffuse, gummatous osteomyelitis. The absence of sequestrums and of evidence of suppuration in both bones was considered an important argument in favor of syphilis.

Raymond and Gangolphe stated that the specimens were examined by Lannelongue, and that they were pronounced syphilitic by him. Gangolphe also said that Lannelongue believed that the hypertrophying osteitis of Paget was a manifestation of syphilis, a view that Gangolphe himself formerly held, but that he no longer entertained.

A brief account of these specimens was given by Pales, with photographs; he offered no personal opinion, but stated that Professor Jeanselme did not appear to agree as to their specific (syphilitic) nature. They were also briefly described by Vorberg, with photographs; his description apparently called the attention of some German observers to them.

Having been quoted by Vorberg as pronouncing the specimens of de Baye syphilitic, Aschoff published a brief note stating his position. He had not seen these bones, but admitted the great similarity of the photographs of them to recent bones known to be syphilitic, of examples of which he gave excellent photographs. He could not give final judgment without personally examining the bones in question. Even then, he said, though there might be the greatest similarity in the findings, that did not prove their identity.

Christeller thought from Vorberg's illustrations that the condition in the de Baye specimens resembled osteodystrophia fibrosa more than it did syphilis.

Weber, working in Aschoff's laboratory, examined various known and doubtful cases of bone disease, as stated in the preceding section of this paper. He concluded that the bones from the Marne (de Baye) were like syphilitic bones, but that they might be from a case of osteodystrophia fibrosa. Study of these bones with the microscope was much to be desired.

The bones of de Baye were studied again in 1930 by Michäelis, who obtained portions of them for examination with the microscope. He concluded that nonspecific osteomyelitis, osteodystrophia fibrosa and the osteo-arthropathy of Pierre-Marie could be excluded. He did not find the mosaic structures of Schmorl, and stated that the participation of the periosteum in the new formation of bone could be seen. (The last is not clearly shown in his photomicrographs.) Michäelis made a diagnosis of syphilis, with such degree of probability as present knowledge permits.

As I have not seen these bones, I am in no position to speak of them with authority. However, I am surprised that so little has been said, by the various observers who have written of them, concerning the large cavity in the middle of the shaft of the ulna, plainly discernible in the photograph of the ulna after it had been sawed, a point in favor of osteodystrophia fibrosa. As far as I can learn from the literature, no roentgenogram of the specimens has been published. Evidently, in the present state of knowledge, no final verdict can be given in this case.

Michäelis described several "bones of the 'stone age'" obtained from the Museum of Saint-Germain-en-Laye, near Paris (fig. 15). Of these, an ulna (his Präparat 30<sup>s</sup>) and a femur (his 31) showed changes that he diagnosed as probable syphilis on the strength of the gross appearance and apparent evidence of periostitis in the section (poorly shown). The condition presented by another femur from the same source (his 34), a fragment, he diagnosed as syphilis by exclusion. His illustrations certainly show new growth of bone from the periosteum. Apparently Schmorl concurred in this opinion. It may be pointed out here that exclusion of osteomyelitis and nonsyphilitic periostitis from such a fragment, in which both ends of the bone were missing, cannot possibly be done to a certainty, or even with probability.

*Other Material, Mainly from France.*—The descriptions of the remaining cases from France are in no way convincing, but the cases are mentioned briefly for the sake of completeness.

The skull of Braye-sur-Seine is to be regarded as an example of the lesion that followed the cauterization practiced in prehistoric times, and fully described by Manouvrier. It was first brought forward as presenting syphilis by Parrot.

Bones and teeth from the dolmens of the Lozère (Cauquenos or Boujassac) excavated by Prunières are not to be regarded as showing ancient syphilis, according to Raymond. They were also presented by Parrot.

A tibia from the dolmen of Maintenon (Eure-et-Loire) was described by LeBaron as having multiple exostoses: The specimen was in the Musée Broca, but appears to have been lost (Raymond, 1911).

A fragment of frontal bone from a tumulus of Méloisy (Côte-d'Or) was described by LeBaron as having two exostoses at the left of the coronal suture, diagnosis uncertain; the specimen cannot now be found. Raymond discovered another skull from the same tumulus with a loss of substance on the frontal bone that he thinks might be due to syphilis.

A tibia from the dolmen of Léry (Eure) was described by LeBaron as his best proof of the existence of ancient syphilis; Raymond appeared to agree with him. The only description given by him is that hyperostosis was present. According to Pales, the specimen seems to have been lost.

The occurrence of these bones in dolmens or tumuli indicates that they probably belonged to the neolithic or to the bronze age; possibly to a later period.

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8. On Michäelis' plate XLVI, the ulna seems to have been incorrectly numbered 28, no doubt through an error of the printer. The arrangement of the illustrations and of the text in this monograph is such that I followed them with difficulty and some uncertainty.

Pales also mentioned among the bones that others had called syphilitic a neolithic femur on which he made the diagnosis of Paget's disease (his plates IV-VI), a parietal bone having osteoporosis (his plate XLIII, fig. 2), and various other bones having lesions that he regarded as not syphilitic. He gave illustrations of a neolithic humerus on which he made the diagnosis of osteitis fibrosa (his



Fig. 15.—“Stone Age” bones. The photograph was furnished by the Museum of St. Germain-en-Laye, France. These bones were discussed by Michaelis; his numbers (from left to right) and diagnosis were: 30, ulna, suggestive of syphilis, 31, femur, suggestive of syphilis; 34, femur (fragment), syphilis.

plates X and XI, roentgenogram), and a neolithic femur and tibia called examples of osteoperiostitis (his plate XXXV), that some might suspect of being due to syphilis.

Saint-Perier described what he believed to be an example of congenital syphilis, of uncertain date but probably of the Gallo-Roman period, found at Souzy-la-

Briche, near Etampes, France. The specimen was the somewhat fragmentary skull of a child, thought to be about 8 years of age. The teeth were not "Hutchinson teeth," but some had fallen out. The pathologic condition consisted of elevated areas on the frontal bones that presented "une apparence criblée"; the same appearance was visible to some extent on the upper surfaces of the orbits and on the right parietal bone. The rest of the skull and such long bones as were found were free from this appearance. From the description and the excellent drawing that accompanies this article, I should be of the opinion that the case was one of symmetrical osteoporosis of the upper surface of the cranium (*cribra parietalia*), and certainly not one of syphilis; however, I have not seen the specimen.

Raymond said further (1911) that no gap exists between prehistoric times and the end of the fifteenth century, because there are some convincing cases of syphilis of bone from the old world of this period. He does not, however, give any details concerning the evidence on which his statement is based.

In another article (1912), Raymond spoke of certain bones presented before the Société de dermatologie in 1894 from a hospital of the Templars found associated with a coin of Verona of the twelfth century. He gave no additional facts.

Spillman described a skull attributed to the Merovingian period, that showed a small depressed scar and had a "worm eaten" appearance; this might have been a healed gummatous lesion or might have been due to traumatism or to cauterization, as he suggested. He gave no illustrations and did not tell where this skull was. He referred to exostoses on the tibias from certain Gallic burials at Nod-sur-Seine, described by Brulard (*Revue préhistorique de l'est de France*, which is not accessible to me).

A case that is supposed to have originated in France, but in which death occurred in Geneva, Switzerland, is mentioned in a brief communication by H. Maillart. In a history of the cathedral of St. Peter, an account is given of the examination in 1869 of the body of a bishop, who came to Geneva from Paris in 1422, dying in 1423. The description of the bones was written by the professor of pathologic anatomy, Zahn. The femur and tibia were stated to show extensive thickening from a combination of osteoporosis and osteosclerosis. The results were pronounced characteristic of syphilis; no illustrations were given. The condition of the cranium was not stated.

Bloch mentioned several specimens in which the lesions were alleged to be those of ancient syphilis: one skull from England, said to be of the time of Richard III, referred to by Carter Blake; a skull from the dolmen of L'Aumede, demonstrated by Broca; various bones from a cemetery on the Rue de Bruxelles in Paris (but of uncertain date), also mentioned by Broca, and (p. 346) a skull of the Merovingian period produced by de Mortillet, and having certain horizontal grooves on the teeth. None of these seems to be of importance, but they are mentioned for the sake of completeness, and because I have not seen references to them in the other authors cited.

In the case of the ancient Swedish King, Magnus Ladulös, reported by Fürst and Olsson (apparently as a case of syphilis), in whom several long bones were involved, Michäelis obtained a portion of the tibia for examination. As this tibia in sections showed undoubted periostitis without severe alterations of the compacta, and as several bones were involved, the condition could not be local periostitis. He regarded it as most probably Pierre-Marie's hypertrophic osteo-arthropathy (Michäelis, pp. 86 and 88; also plates XL-XLIII, all photomicro-



graphs). Professor Sjövall had already published an opinion to the effect that this was probably Pierre-Marie's osteo-arthropathy.

The original articles in Swedish are not accessible to me, and my information is obtained wholly from the monograph of Michäelis.

SYPHILITIC BONES, DATED SOMEWHAT DOUBTFULLY AS ABOUT  
FROM 1500 to 1600 A. D.

It may be impossible to assign exact dates to bones from cemeteries even of historic times. However, some specimens from a time probably near the year 1500 or later show evidence of syphilis, as far as it is possible to make a diagnosis from dried bones.

In his treatise on pathologic anatomy, Lancereaux described and gave drawings of a skull and a femur that appear to be syphilitic. He discussed the possibility that the lesions might have been produced by leprosy, and deplored the lack of information on the condition of the



Fig. 16.—Syphilitic skulls, Paris, Lancereaux, probably of about the year 1500. (Original photograph.)

bones in leprosy. He stated that the specimens were obtained from the catacombs of Paris, but that they came originally from a cemetery on the Rue de Douai, on the site of an ancient leprosarium. Apparently at that date (1889), these catacombs were a promising field for further exploration, likely to yield many specimens showing bone diseases of past centuries.

On a visit to the Musée Dupuytren in 1925, where Professor Roussy gave me access to the splendid material collected there, I found two skulls, which, my notes state, were attributed to Lancereaux, and which came from an old leper cemetery of the fifteenth century. My copy of the label reads "733 and 734, Nouv. Lancereaux. Cimetière dit a Lepreux rue de Douai." My impression of the specimens was that they were as surely syphilitic as it is possible for skulls to be. I obtained fairly good pictures of them with a pocket camera, from which enlargements have been made (fig. 16). Apparently, the character of the

lesions was the same as that shown in Lancereaux's drawing. Whether or not one of them was the subject of his drawing cannot be determined.

Even if it is assumed that the cemetery on the Rue de Douai was used exactly until the year 1500 and no later, the changes in these skulls may have been some of the results of that devastating outbreak of syphilis that occurred at and after the siege of Naples. But the possi-



Fig. 17.—Syphilitic skull, Nuremberg, probably of about the year 1500. The photograph was furnished by Prof. Gustav Hauser, Erlangen.

bility that the lesions were caused by leprosy, as suggested by Lancereaux, might be kept in mind.

The bones found in an ossuary connected with the cathedral of the town of Cham in Bavaria were described by Jäger. Thirty-three skulls showing pathologic conditions had been preserved from some five thousand taken from the ossuary.

Jäger believed that two of them showed the changes of *osteitis tuberosa syphilitica*. The diagnosis was confirmed by von Hansemann, von Luschan and,

for the first specimen, by Kaiserling also. I have not seen these skulls, and it would be unsafe to form an opinion from the illustrations, but the descriptions seem consistent with the diagnosis given. The ossuary of Chammünster was in use during a long period through the Middle Ages, and in a few cases into the nineteenth century. From the evidence available, Jäger believed that the two skulls belonged to about the year 1600. He also described a considerable number of long bones from the same source, on which he made the diagnosis of syphilis (confirmed by von Hansemann), and a skull and a lower jaw from an ossuary at Greding, the descriptions of which are less convincing and which were also less ancient.

Hauser described and discussed an old skull from the cemetery of the Sebaldus church in Nuremberg. The chronicles of the city stated that no burials had taken place after 1519. Hauser thought that the earliest possible record of syphilis in Nuremberg was for the year 1495, and that the date was more probably late in 1496. From these dates Hauser argued that the skull in question belonged to some time between 1497 and 1519. It would appear that the latter date must be accepted, but that the earlier one cannot be determined with certainty.

From Hauser's description of the specimen and from his illustration, it appears that the changes in the skull were probably produced by syphilis (fig. 17).

The lesion was chiefly of the frontal bone and was confined to the outer surface. It consisted of irregular areas of destruction of bone, with new growth of ivory-like bone and some formation of stellate scars, which Hauser believed could have been produced only by syphilitic caries following gummatous periostitis. Hauser found in the Pathological Institute at Erlangen a recent specimen of syphilis of the skull that resembled the Nuremberg skull in all details. He also stated that the syphilitic skulls described by Jäger, referred to on the pages just preceding, were the only other ancient syphilitic skulls from Germany described in the literature.

*(To be Concluded)*

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, etc.**—Ernst Friedberger, director of the Forschungs-Institut für Hygiene und Immunitätslehre in Berlin-Dahlem, outstanding teacher and investigator in the field of immunology and an editor of *Zeitschrift für Immunitätsforschung und experimentelle Therapie* since its beginning, has died at the age of 57.

Ward J. MacNeal has been appointed professor of pathology and bacteriology in the faculty of the New York Post-Graduate Medical School and Hospital of Columbia University.

It is announced that the chair of medicine in the College of France, made vacant by the retirement of Professor D'Arsonval has been filled by the appointment of Charles Nicolle, director of the Pasteur Institute of Tunis and recipient of the Nobel Prize in medicine for 1928 for his work on typhus fever.

Milton C. Winternitz, dean and professor of pathology in the school of medicine of Yale University, has been awarded the Jenkins medal for meritorious service to the dental profession by the Connecticut State Dental Association.

**Society News.**—The Buffalo Pathological Society has been organized with Kornel Terplan as president and William Jacobs as secretary.

At its recent meeting in Philadelphia the American Association of Immunologists elected A. B. Wadsworth president and Arthur F. Coca secretary-treasurer.

The recently elected officers of the American Association for Cancer Research are: president, E. B. Krumbhaar; vice-president, Ward J. MacNeal; secretary-treasurer, William H. Woglom.

G. R. Callender has been elected president, Victor C. Jacobsen vice-president and Maude E. Abbott secretary-treasurer of the American and Canadian Section of the International Association of Medical Museums.

# Abstracts from Current Literature

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## Pathologic Anatomy

A RARE OSSEOUS DYSTROPHY (MORQUIO). HERMAN F. MEYER and JOSEPH BRENNEMANN, *Am. J. Dis. Child.* **43**:123, 1932.

A child is described, with a peculiar, gross, generalized bodily deformity complex that is unique in our experience. We have been able to find only one report in the literature of an apparently identical condition. The essential pathologic change is osseous dystrophy involving practically the whole skeleton except the head, in which achondroplasia is an important factor. We believe that the condition is a definite, uniform clinical entity, that is, *sui generis*, and we hope that this view may be confirmed or refuted in the experience of others.

AUTHORS' SUMMARY.

HODGKIN'S DISEASE OF BONE MARROW AND SPLEEN WITHOUT APPARENT INVOLVEMENT OF LYMPH NODES. E. B. KRUMBHAAR, *Am. J. M. Sc.* **182**: 764, 1931.

A fatal case of Hodgkin's disease with autopsy is reported that was practically limited to the spleen and bone marrow, though in these two organs the involvement was extensive. The histologic appearance of the femoral [marrow] suggested that the disease process might have been primary in this tissue. No involvement of lymph nodes could be found, and the lymphoid tissue of the gastrointestinal tract was histologically unchanged. While this does not constitute unequivocal proof of the absence of involvement of the lymph nodes, it points strongly in this direction; while the absence of lesions in the mucosae and of the so-called germinal centers in the spleen suggests interesting speculations as to the type of cell (reticular or endothelial) in which the disease process originates. It is suggested that the disease should be considered a disease of the hematopoietic or of the reticulo-endothelial system rather than of lymphoid tissue.

AUTHOR'S SUMMARY.

MYELOID CELL HYPERPLASIA OF THE BONE MARROW IN AGRANULOCYTIC ANGINA. THOMAS FITZ-HUGH and E. B. KRUMBHAAR, *Am. J. M. Sc.* **183**: 104, 1932.

Myelocytes and myeloblasts were found in the bone marrow at necropsy in more than normal numbers in a case of typical "agranulocytic angina" the ante-mortem blood count in which was 200 white cells per cubic millimeter (all lymphocytes); i. e., a marked absolute reduction of lymphocytes and absence of all other white cells. Based on this and similar cases recorded in the literature, objection is raised to the current hypothesis of "granulocytic aplasia" as constituting the "primary" pathologic mechanism of the disease, and in its place a hypothesis of "maturation arrest" is proposed for consideration and future study. As there is an absolute reduction of lymphocytes in the blood stream as well as of neutrophils, and on account of certain analogies with pernicious anemia, a designation such as pernicious leukopenia is suggested as preferable to the more widely used names for this disease.

AUTHORS' SUMMARY.

PULMONARY FIBROSIS: EXPERIMENTS OF SHORT DURATION. WILLIS S. LEMON and GEORGE M. HIGGINS, *Am. J. M. Sc.* **183**:153, 1932.

It seems probably that the fibrosis of the lungs produced in workers exposed to dusty atmospheres, especially those in which fine particles of silica make up

the larger percentage of the dust, is an end-product and represents a progressive defeat of the protective mechanisms of the body. It is never found until the lung fails to rid itself of dust carried out of bronchi by ciliary action or of dust carried through the lung by way of the lymphatic channels. With the breakdown in the carrying mechanisms, the burden of protection is on the phagocytic cell, which, with its load of engulfed material, passes to all parts of the lung and ultimately becomes immobilized in those portions of parenchyma in which lymphatic tissue is abundant. The cells concerned are polymorphonuclear leukocytes, which are the first to appear, to disintegrate and to disappear, and clasmatocytes, which appear later, are extremely active, break up less readily, and may become transformed into fibroblasts. In association with the tissue fibroblasts the clasmatocytes form scar tissue. Finally, fibrosis is the result of the action of substances secreted by the cell on the foreign particles. If a tissue poison results from this chemical action, fibrosis is encouraged, and is progressive, lasting as long as the unaltered irritant remains in the lung

AUTHORS' SUMMARY.

THE INCIDENCE AND SITUATION OF MYOCARDIAL INFARCTION IN ONE THOUSAND CONSECUTIVE POSTMORTEM EXAMINATIONS. ARLIE R. BARNES and RALPH G. BALL, *Am. J. M. Sc.* **183**:215, 1932.

In 1,000 unselected consecutive postmortem examinations more or less localized myocardial infarction was recognized grossly in 49 subjects (4.9 per cent). Of 685 subjects 40 years of age or more, 47 (6.86 per cent) presented myocardial infarction. A majority of the subjects who had sustained myocardial infarction had had associated hypertension, as judged by the cardiac weights and the records of blood pressure. Notable preponderance of arteriosclerosis in the left coronary artery over that found in the right was not observed in the hearts in which evidence of infarction was found. Gross myocardial infarction resulting from coronary occlusion was practically confined to the left ventricle. Myocardial infarction was observed in the posterior basal portion of the left ventricle in 24 instances as compared with 28 instances in which infarction involved the apex and anterior portion. More careful pathologic study of the posterior basal portion of the left ventricle is urged in order that infarctions in that region may not be overlooked. In 28 instances, infarction occurred in the region supplied by the anterior descending branch of the left coronary artery, as compared with 20 instances in which it occurred in the region of the left ventricle supplied by the right coronary artery. The designation of the anterior descending branch of the left coronary artery as "the artery of coronary occlusion" is no longer justifiable.

AUTHORS' SUMMARY.

DISSECTING ANEURYSMS. M. D. TYSON, *Am. J. Path.* **7**:581, 1931.

The development of a dissecting aneurysm of the aorta is apparently dependent on degenerative changes in the medial coat. The underlying cause of the medial change is probably obliteration of a large number of vasa vasorum from arteriosclerosis or from a low grade inflammatory process. The aneurysm begins by a rupture of one or more vasa vasorum into the weakened medial layer, with the formation of a hematoma which splits apart the medial fibers. A tear in the intima of the aorta is not a necessary factor in the formation of a dissecting aneurysm. When intimal tears occur, they are probably secondary to the development of the aneurysm.

AUTHOR'S SUMMARY.

MELANOSIS MUCOSAE APPENDICIS VERMIFORMIS. R. D. LILLIE, *Am. J. Path.* **7**:701, 1931.

Melanosis of the appendix resembles essentially the melanosis coli described by Pick and others, and probably often forms a part of that condition. The pigment is probably formed first as fine, uncolored granules in large mononuclear cells immediately beneath the surface epithelium. The pigment-carrying cells appear to

be macrophages and fibroblasts. The average age of patients with pigmented appendixes is slightly greater than that of patients with nonpigmented appendixes. No especially significant differences in incidence were found on segregation according to race, sex or occupation. The great diminution in frequency of melanosis in acute and obliterative appendicitis, while undoubtedly due in part to mucosal destruction, is probably not due entirely to that factor, as nonobliterative, non-ulcerative subacute appendicitis shows a similar though less marked decrease in the incidence. Adhesions have no noteworthy influence on the incidence of melanosis; atrophy of lymph follicles is possibly associated with a slight increase, and dilatation is quite definitely correlated with an increased frequency and probably a higher grade of melanosis. The duration of symptoms of chronic appendicitis appears to be definitely greater in melanotic than in nonmelanotic appendixes. It is noteworthy that the frequency of melanosis reported here is greater than it has been stated to be in any other published reports on melanosis coli, and the association with chronic appendicitis with dilatation and of longer than average duration may indicate that such chronic appendicitis provides favorable conditions for increased deposition of the pigment. *AUTHOR'S SUMMARY.*

MAST MYELOCYTE LEUKEMIA IN A CAT. R. D. LILLIE, *Am. J. Path.* 7:713, 1931.

Of some twelve reported or mentioned cases of leukemia in cats, this is the fourth to be recorded as splenic or myelogenous and the first in cats in which basophilic myelocytes have been the predominating cell type. About nine cases of mast myelocyte leukemia have been reported in man. Anatomically, this case presented an enormous enlargement of the spleen, enlargement of the liver and extensive mast myelocyte infiltration of the splenic pulp, mast myelocyte nodules in the liver and distention of hepatic capillaries by blood and large numbers of mast myelocytes. Numbers of mast myelocytes were seen in hepatic and renal veins. The blood, lymph glands and bone marrow were not examined.

*AUTHOR'S SUMMARY.*

RENAL LESIONS IN THE TOXEMIA OF PREGNANCY. E. T. BELL, *Am. J. Path.* 8:1, 1932.

In fatal cases of eclampsia a characteristic glomerular lesion is found. The glomeruli show marked narrowing of all the capillaries, caused by an increase in thickness of the basement membrane of the capillary, but sometimes by an increase of endothelial cells. One case is reported in which the lesions resulting from an attack of eclampsia seven years before are described. These consist of focal hyaline areas in the glomeruli with partial or complete glomerular obliteration and varying degrees of tubular atrophy. It is shown that a peculiar form of chronic renal disease may result from the eclamptic kidney. In one case of hyperemesis gravidarum, glomerular lesions were found that corresponded entirely to those of typical eclampsia. In three other cases, the glomeruli were normal. A fatty liver without necroses is characteristic of this form of toxemia. When a woman with chronic renal disease becomes pregnant, there is usually an aggravation of all the nephritic symptoms. The condition cannot be distinguished from preeclampsia and eclampsia unless the condition of the kidneys prior to pregnancy is known, or unless there is a definite impairment of renal function. In women with chronic nephritis there is shown no special tendency toward the development of eclampsia during gestation.

*AUTHOR'S SUMMARY.*

TULAREMIC ENCEPHALITIS WITH COEXISTING TUBERCULOSIS. F. W. HARTMAN, *Am. J. Path.* 8:57, 1932.

A case of acute tularemia with diffuse encephalitis and coexisting tuberculosis of the kidney and epididymis is described. The lesions of the brain are readily

seen as areas of yellow necrosis and hemorrhage throughout the base. Microscopically, there is patchy necrosis with infiltration by round, wandering cells and polymorphonuclears.

AUTHOR'S SUMMARY.

THE FREQUENCY OF ANOMALOUS RETICULA (CHIARI NETWORK) IN THE RIGHT ATRIUM OF THE HUMAN HEART. F. C. HELWIG, *Am. J. Path.* 8:73, 1932.

Eight cases of Chiari's network are recorded. In the first four cases, severe cardiac lesions were found which were the direct cause of the patient's death. In the last four cases, the findings in the heart, aside from the Chiari network, were negligible, and in none of the entire group could any of the clinical symptoms be ascribed to the network itself. Although so few cases have been reported, such reticula are not particularly rare in the human heart, since they were found to be present in 1.5 per cent of routine autopsies. These structures are easily destroyed by careless manipulation, and no doubt will be found quite often if more care is exercised in removing and opening the heart. They were of no clinical significance in this series of cases.

AUTHOR'S SUMMARY.

SYPHILITIC AORTIC ENDOCARDITIS AND SUPERIMPOSED STREPTOCOCCUS VIRIDANS ENDOCARDITIS. E. B. CRAVEN, *Am. J. Path.* 8:81, 1932.

In summary, the opinion that in the case reported here the changes in the aorta and aortic valve were basically syphilitic, with a superimposed bacterial endocarditis, is supported by the following facts: lack of a history of articular rheumatism; a history of primary and secondary syphilitic lesions; a strongly positive Wassermann reaction of the blood; absence of lesions of rheumatic fever in the pericardium, myocardium and mitral valve; large and prominent perivascular infiltrations of the aortic adventitia, composed almost exclusively of small lymphocytes; large aortic vascular medial scars penetrating deeply into the medial coat, with the formation of new vascular channels; an acute exudate composed of fibrin, leukocytes and gram-positive cocci (*Streptococcus viridans*) on the surface of the aortic valve; identification of *Streptococcus viridans* not only in a culture of the vegetations on the aortic valve, but also in cultures from the blood.

AUTHOR'S SUMMARY.

FORMATION OF BONE MARROW IN THE SUPRARENAL GLAND. D. C. COLLINS, *Am. J. Path.* 8:97, 1932.

Fifteen instances of formation of bone marrow in the suprarenal gland have been collected from the literature, with a compilation of the salient features. They have been classified according to Soos' method. Theories concerning the mechanism of their origin have been discussed. An additional example has been reported from the Mayo Clinic with illustrations of the salient gross and microscopic appearances.

AUTHOR'S SUMMARY.

MULTIPLE INFARCTS AND NECROSES OF THE SPLEEN (FLECKMILZ). G. RAKE, *Am. J. Path.* 8:107, 1932.

As far as may be told from examining the changes in the spleen, the sequence of events in the cases described seems to be somewhat as follows: Some toxic or other process leads to endarteritis obliterans of the larger and medium-sized vessels and to a hyaline change of the smaller vessels (arterioles) in the kidneys and spleen. At a later date this process becomes more acute, with fulminant necrosis of the walls of the vessels and obliteration of the medium-sized and smaller arteries by means of thrombi. Meuret came to identical conclusions in regard to the two cases that he studied. Owing to the number of vessels involved, the blood supply to areas of the spleen is shut off or reduced below the minimal amount required for the maintenance of the vitality of the tissues, so that necrosis



occurs. At the same time this exacerbation of the arterial and arteriolar disease seems to coincide, if it is not identical, with the factors that lead finally to the death of the patient. Death therefore results a short time after the onset of the necroses in the spleen, which, for this reason, are mostly fresh and show only very early organization at the margin.

AUTHOR'S SUMMARY.

KÖHLER'S DISEASE OF THE SCAPHOID BONE. C. VELLUDA and M. I. NICHITA, *Virchows Arch. f. path. Anat.* **276**:548, 1930.

The authors believe Köhler's disease of the tarsal scaphoid bone to be the result of diminished blood supply to the bone. The diminished blood supply may be due to maldevelopment or arrested development of the supplying vessels or to thrombosis or traumatic involvement of the vessels. The arterial supply of the scaphoid bone is through the scaphoid artery, which is a branch of the dorsalis pedis artery. The distribution of the artery was studied in roentgenograms made after arterial injections of bismuth. In five of ten preparations the scaphoid artery did not have any anastomoses before entering the bone. The authors conclude that such an arterial supply would predispose to Köhler's disease.

W. SAPHIR.

LEUKEMIC RETICULO-ENDOTHELIOSIS. H. E. BOCK and K. WIEDE, *Virchows Arch. f. path. Anat.* **276**:553, 1930.

This is a clinical and pathologic report of a case that ran the typical course of acute leukemia. The leukocyte count increased progressively from 10,000 to 46,000, and there was a rapid increase of monocytes to 85 per cent. The authors accept von Schilling's view that the monocytes are derived from the reticulo-endothelial system. They therefore conclude that their case of monocytic leukemia was an example of leukemic reticulo-endotheliosis.

W. SAPHIR.

THE STRUCTURAL LESIONS OF DIABETIC COMA. I. I. MEDWEDEFF, *Virchows Arch. f. path. Anat.* **276**:622, 1930.

From examination of nine patients dying in diabetic coma, Medwedeff describes the characteristic changes as glycogenic infiltration of the organs, especially of the liver and kidneys; alterations in the islands of Langerhans, such as fibrosis, atrophy and formation of hypertrophied cells with large, pale nuclei; shrinkage of ganglion cells, distention of periganglionic lymph spaces, fatty change and nuclear alterations in the vegetative nervous centers (globus pallidus, caudate nucleus and hypothalamus).

W. SAPHIR.

EFFECT OF ROENTGEN IRRADIATION ON CALLUS FORMATION. S. FUKASE, *Virchows Arch. f. path. Anat.* **277**:69, 1930.

The action of roentgen irradiation on the formation of callus and union was studied experimentally in eighty-four rabbits. The fibula of each side was fractured. The fracture of one leg was irradiated, that of the other being left untreated as a control. Intensive irradiation led to decreased formation of callus and delayed union of the bone. Less intensive irradiation led to diminution in formation of callus and bony union within a shorter period as compared with the control side. The amount of preliminary callus formed was less, and its calcification occurred earlier in the treated than in the untreated fracture. Microscopically, there were increased calcification and diminished vascularization and connective tissue formation.

W. SAPHIR.

ARTERIAL CHANGES DUE TO IRRADIATED STEROL. ALICE SCHIFF, Virchows Arch. f. path. Anat. **278**:62, 1930.

Irradiated oil was administered to cats and rabbits in daily doses of from 0.8 to 4 mg. for periods varying from a few days to four months. Larger doses over a prolonged time caused progressive cachexia and wasting. The arterial changes, which involved primarily the media, were most marked in the aorta. The carotid arteries were more frequently damaged than the iliac. In the cat, the arteries of the heart and lung were involved more often than those of the kidney; in the rabbit, the renal arteries were more susceptible. The earliest change noted was swelling of the ground substance of the media. This was followed by irregularity in the course of the elastic fibrils, granular degeneration of the ground substance, rupture of the collagen fibers, deposition of calcium and histiocytic proliferation. The process may involve the circumference of the vessel or may be limited to localized patches. Slight intimal proliferation and moderate cellular infiltration of the adventitia were occasionally seen. The lesions do not regress or heal spontaneously following cessation of administration of the oil.

O. T. SCHULTZ.

### Pathologic Chemistry and Physics

BLOOD LIPASE, DIASTASE AND ESTERASE IN MULTIPLE SCLEROSIS. L. A. CRANDALL, JR., and IAN S. CHERRY, Arch. Neurol. & Psychiat. **27**:367, 1932.

Crandall and Cherry studied the blood of patients suffering from multiple sclerosis for the presence of lipase, esterase and diastase. Seventy-eight per cent of patients with multiple sclerosis show the presence of lipase, as against 76 per cent of controls and 80 per cent of patients with a lesion of the liver. Diastase was found in 47.6 per cent of patients with multiple sclerosis and in 75 per cent of those with disease of the liver, while esterase was present within normal limits.

GEORGE B. HASSIN.

THE NATURE AND ORIGIN OF SYNOVIAL FLUID. D. H. KLING, Arch. Surg. **23**:543, 1931.

Normal synovial fluid in about 95 per cent of the effusions in the joints gives precipitation phenomena with dilute acetic acid, hydrochloric acid and mercuric chloride. These reactions are not found in plasma transudates and exudates in other areas of the body, but they do occur with saliva and egg white. The viscosity of synovial fluid is higher than that of plasma and exudates. The specific gravity is in the range of that of other body fluids. A mucinous substance was prepared from the effusions of synovial membranes, which seems to be responsible for the physical chemical properties of this fluid. Mucinous granules were demonstrated in the active cells.

N. ENZER.

KETONE EXCRETION. J. A. BEHRE, J. Biol. Chem. **92**:679, 1931.

The concentration of acetone and diacetic acid in the urine is normally less than 0.5 mg. per hundred cubic centimeters. The amount excreted is highest during the afternoon and evening. During the night there is a decrease in both the amount and the percentage excreted. A still greater decrease in the percentage concentration occurs during the morning, although the actual amount excreted per hour is greater than at night. The increased amount is associated with an increase in the urinary volume and may represent a "washing out" of substances formed during the night. In the early stages of fasting, an increased excretion of ketone bodies is observed as early as from six to twelve hours after the ingestion of food. The excretion of acetone and diacetic acid by diabetic persons receiving

insulin increases during the night, reaches a maximum in the morning and decreases gradually to a minimum, which occurs during the six hours following the last insulin treatment of the day. The concentration remains above the normal level, even when the excretion of sugar is kept normal by insulin.

E. R. MAIN.

CALCIUM AND PHOSPHORUS INTAKE IN HYPERCALCEMIA AND HYPERPHOSPHATEMIA DUE TO IRRADIATED ERGOSTEROL. J. H. JONES and M. RAPPORT, *J. Biol. Chem.* **93**:153, 1931.

The administration of viosterol to dogs maintained on a calcium-free diet produces hypercalcemia. Calcium chloride produces a slight increase in the blood calcium and a more marked effect if viosterol is administered forty-eight hours before. The administration of disodium phosphate produces marked hyperphosphatemia both before and after administration of viosterol. The administration of calcium and phosphorus together, as an aqueous solution of dicalcium phosphate, produces a slight decrease in the calcium and almost no change in the phosphorus content of the blood. Following the administration of viosterol the calcium becomes increased, while the phosphorus level tends to remain constant.

E. R. MAIN.

DIFFUSIBILITY OF THE PROTEINS OF NORMAL AND PATHOLOGIC PLASMA. O. H. GAEBLER, *J. Biol. Chem.* **93**:467, 1931.

Experiments on the dialysis of plasma proteins with collodion membranes of approximately equal permeability in both directions were without evidence that proteinuria is due to an increase in the diffusibility of the proteins or to the presence of substances increasing the permeability of the membranes.

E. R. MAIN.

CALCIFICATION OF THE TIBIA OF THE NEWBORN CHILD. L. E. BOCHER and G. H. HANSMANN, *J. Biol. Chem.* **94**:195, 1931.

The average percentages of ash and calcium in the dry, fat-free tibias of newly born, normal infants are 44.4 and 16.6, respectively. The inorganic composition of these bones appears to be almost invariant. It was unaffected by large differences in the dietary intake of the mothers.

A. P. LOCKE.

THE RELATION OF HYDROSTATIC PRESSURE TO THE GRADIENT OF CAPILLARY PERMEABILITY. P. D. McMASTER, S. HUDACK and P. ROUS, *J. Exper. Med.* **55**:203, 1932.

The gradient of permeability along the capillaries of voluntary muscle and the capillaries and venules of skin exists independently of the hydrostatic conditions, though influenced by them. Its presence cannot be explained by a graded tonic contraction of the capillaries. The evidence, like that of previous papers, points to local differences in the barrier offered by the walls of these vessels as responsible for the gradient.

AUTHORS' SUMMARY.

BIOLOGIC ACTION OF ULTRAHIGH FREQUENCY CURRENTS. W. T. SZYMANOWSKI and R. A. HICKS, *J. Infect. Dis.* **50**:1, 1932.

Ultrahigh frequency irradiation under the described conditions is capable of producing definite attenuation of the three major bacterial toxins, diphtheria, tetanus and botulinus, in raw broth filtrates. This effect is obtained without the development in the toxin of temperatures that would by themselves affect the potency of the toxin. A tentative theory is advanced pertaining to the mechanism of this action.

AUTHORS' SUMMARY.

ADSORPTION OF TUBERCULIN BY COAL DUST. S. LYLE CUMMINS and C. WEATHERALL, *J. Hyg.* **31**:464, 1931.

Anthracite coal dust is capable of adsorbing the active principle of tuberculin. This power of adsorption is especially well marked when the contact between the coal dust and the tuberculin is brought about in a slightly alkaline medium. Previous saturation with serum proteid, while it appears to diminish, does not annul the power of coal dust to adsorb the active principle of tuberculin.

AUTHORS' SUMMARY.

MALARIAL PIGMENT. F. ROCCHI, *Arch. ital. di anat. e istol. pat.* **1**:613, 1930.

Malarial pigment was obtained from the spleen by Warasi's method. The pigment was soluble in normal human plasma, in which clotting was prevented by the addition of hirudin. The pigment itself, as well as its ash, gave the iron reaction by Seyfarth's method, which suggests a hematogenous origin.

G. PATRASSI.

MICRODETERMINATION OF LECITHIN IN BLOOD AND PLASMA. W. LINTZEL and G. MONASTERIO, *Biochem. Ztschr.* **241**:273, 1931.

A method for the determination of lecithin from 10 cc. of blood or plasma is described which is based on the determination of the choline bound to the phosphatide, and which is accomplished by the titration of the trimethylamine freed from this compound. The lecithin content of the plasma is always somewhat lower than that of the total blood. The lecithin content of human blood varies from 0.165 to 0.148 mg. per hundred cubic centimeters, and that of plasma, from 0.141 to 0.121 mg. per hundred cubic centimeters. Sphingomyeline is included in the lecithin figure, but the error from this is small, because sphingomyeline occurs in negligible amounts in the blood.

WILHELM C. HUEPER.

IODINE AND BROMINE CONTENT OF THE THYROID GLAND. F. TANINO, *Biochem. Ztschr.* **241**:392, 1931.

The water in thyroid glands with little colloid is considerably more than in glands with moderate or high colloid. The ratio of bromine to iodine is increased in thyroid glands containing much colloid (quotient from 16.2 to 15.7) over that in glands with a small amount of colloid (quotient 8.3). The glands from exophthalmic goiters treated with iodine and often with bromine before removal show a low bromine and a high iodine content (quotient below 1). The accumulation of iodine in the gland counteracts apparently the storage of bromine. Examination of the colloidal material gave markedly varying values of iodine and bromine. It appears certain that the high bromine-iodine quotient in thyroid glands with much colloid does not depend on the amount of these substances present in the colloid.

WILHELM C. HUEPER.

CHANGES PRODUCED IN THE REDUCING SUBSTANCES OF THE BLOOD BY IRRADIATION. SOITIRO YOKOTA, *Biochem. Ztschr.* **241**:398, 1931.

The amount of reducing substances in the blood is changed by irradiation. By the method of Salkowski the blood sugar proper was separated from the other reducing substances. In animals irradiated with ultraviolet rays, a decrease in the amount of reducing substances not precipitable by copper-calcium treatment and a relative increase of the precipitable ones were observed. This cannot be due to changes in the glycoposphates. But, as the amount of phosphorus decreased proportionally more in the filtrate after irradiation than in the blood after irradiation.

tion, other phosphorus containing substances must be responsible for the changes observed. Under the effect of irradiation an increase of the phosphorus containing substances precipitable by the copper-calcium treatment occurred.

WILHELM C. HUEPER.

THE INFLUENCE OF INFECTIOUS PROCESSES ON THE PERMEABILITY OF ERYTHROCYTES. M. N. FISCHER and E. A. WOLKOWA, Frankfurt. Ztschr. f. Path. **41**:285, 1931.

The authors examined the permeability of ions within the erythrocytes in a case of typhoid fever. They summarize their findings as follows: The ions of the inorganic ionically dispersed crystalloids diffuse in and out of the erythrocytes. In this process, the Donnan equilibrium of the ions between the red blood cells and the plasma that is normally present is changed in such a manner that the equilibrium constant at the corresponding  $p_H$  concentration is smaller than under normal conditions. There occurs a diffusion of the  $Na^+$ ,  $H^+$  and  $HCO_3^-$  ions into the inside of the erythrocytes and of the  $Cl^-$  ions out of the erythrocytes, with a loss of alkaline reserve (of the bicarbonates) in the plasma. These are synergetic processes, which tend to keep the hydrogen ion concentration constant. Considerable changes in the amounts of sugar and "rest" nitrogen within the erythrocytes cannot be explained by the authors and call for further research.

O. SAPHIR.

POSTMORTEM CHANGES IN SPINAL FLUID. S. SÜMEGI and L. FINDEISEN, Frankfurt. Ztschr. f. Path. **41**:431, 1931.

The colloid reaction of the spinal fluid removed post mortem reveals a normal curve until about nine hours after death. Later, the precipitation zone shifts to the acid side. A few hours after death, there is an increase of the protein and nonprotein nitrogen content of the spinal fluid. The albumin-globulin ratio is smaller than normal. Dextrose can be demonstrated in the spinal fluid a few hours after death. The  $p_H$  of the spinal fluid shifts very soon toward the acid side.

O. SAPHIR.

EFFECT OF EPINEPHRINE ON THE IODINE CONCENTRATION OF THE BLOOD. A. SCHITTENHELM and B. EISLER, Klin. Wchnschr. **11**:9, 1932.

On intravenous injection of epinephrine hydrochloride there is a sudden increase of iodine in the blood in normal adults and in patients with myxedema. On injection of epinephrine hydrochloride into patients with exophthalmic goiter, in whom there is a high concentration of iodine in the blood, the iodine drops below normal. Explanation of these reactions is not offered.

EDWIN F. HIRSCH.

QUANTITATIVE DETERMINATION OF COPPER IN TISSUES. E. CHERBULIEZ and S. ANSBACHER, Virchows Arch. f. path. Anat. **278**:365, 1930.

The authors give a succinct description of a titrimetric method for the determination of copper in animal tissues and organs. They claim that the method will detect 0.001 mg. of copper, and that it is accurate to within plus or minus 0.0005 mg.

O. T. SCHULTZ.

THE ELECTRICAL CHARGE OF LEUKOCYTES IN INFLAMED TISSUES. A. D. ADO, Ztschr. f. d. ges. exper. Med. **79**:752, 1931.

Leukocytes always retain their negative charge after their entrance into inflamed tissues. The height of the potential of the leukocytes in an exudate is much lower than that of the leukocytes in the circulating blood, and their rapidity of electrical discharge is less. When leukocytes taken from exudates are dispersed

in another medium, such as blood serum, physiologic solution of sodium chloride, etc., their electrical potential is decreased in the same degree as that of leukocytes in the circulating blood. Phagocytic leukocytes have a lower electrical potential than normal ones from the blood.

AUTHOR'S SUMMARY.

THE CHANGES IN BLOOD VOLUME IN EXPERIMENTAL SHOCK. W. GRUNKE and W. HARING, *Ztschr. f. d. ges. exper. Med.* **79**:763, 1931.

The usefulness of the dye method for the determination of blood volume in conditions of circulatory collapse has been proved in a series of experiments on dogs. It was determined that although the distribution of the dye in various parts of the circulatory system is approximately the same, the concentration of the dye in the blood falls more rapidly in a condition of shock than in normal blood. Determinations of the blood volume in rabbits in shock showed a diminution of from 30 to 50 per cent below the normal. The lessening of the blood volume in the shock induced by diphtheria toxin varied between 1.1 and 15.2 per cent. Only in one case did the volume fall as much as 31.2 per cent. To explain the results of the latter experiments the authors declare that it is not so much a matter of central or peripheral vasodilatation as it is of changes in the heart action.

AUTHORS' SUMMARY.

SURFACE TENSION OF BLOOD SERUM. WALTER SAUER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:450, 1931.

The surface tension of serums from patients with various manifestations of syphilis showed no characteristic differences from the normal. The previously reported lowering of the tension in persons older than 50 years could not be confirmed, but lowering was constant in inactivated and hemolyzed serums. Repeated estimations of surface tension in the same samples of normal serums gave constant readings, while marked variations were observed in samples of serum from patients suffering from mental diseases. The ring method of Brinkman was employed.

I. DAVIDSOHN.

ANTIRACHITIC VITAMIN FROM IRRADIATED ERGOSTEROL. A. WINDAUS and A. LUETTINGHAUS, *Ztschr. f. physiol. Chem.* **203**:70, 1931.

Two antirachitic, highly effective compounds, vitamins D<sub>1</sub> and D<sub>2</sub>, were isolated in crystalline form from the irradiation products of ergosterol. The vitamin D<sub>1</sub> was obtained in from 30 to 40 per cent by irradiation of ergosterol through a uvioilglass filter, which is penetrated by rays above 290 millimicrons. The vitamin D<sub>2</sub> was isolated by Linsert in magnesium spark preparations, yielding up to 30 per cent of the irradiated substance as vitamin D<sub>2</sub>. The vitamins D are isomeres of ergosterol. They are very stable over many months, if they do not come in contact with air. Both vitamins are toxic, if given in excessive amounts.

WILHELM C. HUEPHER.

### Microbiology and Parasitology

ELECTROPHORESIS OF ORGANISMS BELONGING TO THE PNEUMOCOCCUS AND DIPHTHERIA GROUPS. RANDALL L. THOMPSON, *Am. J. Hyg.* **14**:235 and 244, 1931.

No relationship was found to exist between the virulence of a pneumococcus strain for white mice and the electrophoretic rate of migration of that strain. The electrophoretic rate of migration of the three pneumococcal types decreases in the order: III, I and II. Most strains of organisms belonging to the pneumococcus group may be included in one of three large electrophoretic groups. The existence of two other such groups has been suggested; one of these has not been shown to be definitely distinct from one of the first groups, and the second contains

an insufficient number of members to be significant. All typical strains of any one serologic type of pneumococcus probably belong to the same electrophoretic group. However, one electrophoretic group may include strains not classified in the same serologic category. Rough strains of pneumococcus may or may not migrate more slowly in the electric field than their parent or other strains of the same type as the one from which they were derived.

Both the Abramson and Mudd electrophoresis assemblies were found to give reliable results in the electrophoresis of diphtheria bacilli. The results derived from a study of 173 cultures belonging to the diphtheria group of organisms indicate that toxigenic and nontoxigenic cultures may generally be separated by the method of electrophoresis. The toxigenic organisms migrated more slowly than the nontoxigenic organisms.

AUTHOR'S SUMMARIES.

EARLY LESIONS OF AMOEBIASIS IN MAN, MONKEYS AND CATS. H. L. RATCLIFFE, *Am. J. Hyg.* **14**:337, 1931.

A comparison of the lesions in monkeys with those described in human intestinal amebiasis shows that they are similar and probably originate in much the same way. The presence of amebas deep in the tissues of man may be accounted for by postmortem invasion, since descriptions are made from tissue fixed several hours after death. The lesions of intestinal amebiasis in the cat do not appear to be comparable to those in primates. In the cat, the pathologic process is one of direct invasion and destruction of the wall of the intestines by the amebas. This is usually accompanied by an inflammatory reaction of the whole wall, indicating bacterial as well as amebic action. True undermining ulcers, comparable to those in primates, probably do not occur, and lesions presented as such seem to have another explanation.

FROM AUTHOR'S SUMMARY.

EXPERIMENTAL TRANSMISSION OF EPIZOOTIC FOX ENCEPHALITIS. R. G. GREEN, N. R. ZIEGLER, E. T. DEWEY and J. E. SHILLINGER, *Am. J. Hyg.* **14**:353, 1931.

Epizootic encephalitis of foxes has been transmitted to experimental animals from foxes dying during epizootics occurring in two successive years in the Fromm fur ranges. The inoculation of brain and spinal cord virus by cistern puncture into foxes raised under quarantine conditions has been found to be a successful means of maintaining the encephalitis virus. In the experiments here reported, virus stored in 50 per cent glycerin for five and a half months appears to have the same pathogenic properties as does fresh virus. Intramuscular injection of brain virus appears to transmit the disease almost as uniformly as does inoculation by cistern puncture. The spleen of a fox dying of the experimental disease is very active in transmitting the infection to young foxes by cistern puncture. The disease has also been transmitted by the injection of spleen virus into the peritoneal cavity. The virus has been demonstrated in the heart blood of a fox dying of the experimental infection. We have failed to transmit the disease to five foxes by corneal scarification, even though the scarification was thorough and an experimental virus known to be virulent was used. The disease has also been transmitted to young experimental foxes by skin scarification, by intratesticular injection and by inoculation into the nasal cavity. While the mortality of the natural disease seldom appears to exceed 20 per cent of those exposed, the mortality of experimentally inoculated young foxes is approximately 70 per cent.

AUTHORS' SUMMARY.

AVIAN TUBERCULOSIS IN NORMAL AND VACCINATED RABBITS. E. M. MEDLAR, *Am. J. Path.* **7**:475, 1931.

There is a significant difference in the gross pathology and in the histopathology of nonvaccinated and vaccinated rabbits intravenously inoculated with virulent

avian tubercle bacilli. The textbook description of the tubercle—"a collection of epithelioid cells set in a reticulum with a giant cell in the center and a tendency to undergo caseation"—represents a retrogressive, not a progressive, phase of the pathology of tuberculosis. There is a striking difference in the leukocytic reaction of nonvaccinated and vaccinated rabbits intravenously infected with virulent avian tubercle bacilli. The leukocytic response in the vaccinated rabbits simulates very closely the leukocytic reaction we have observed in human beings who have progressive tuberculosis. This reaction is not specific for tuberculosis, but is caused by unmixed tubercle bacillus infection. The megacaryocyte plays an important rôle in acute avian tuberculosis in the rabbit. What the real significance of the participation of the megacaryocyte in acute tuberculosis is cannot be stated at present.

AUTHOR'S SUMMARY.

LESIONS SUGGESTIVE OF HODGKIN'S DISEASE IN TUBERCULOSIS. E. M. MEDLAR and K. T. SASANO, *Am. J. Path.* 7:491, 1931.

Lesions resembling Hodgkin's disease are often present in acute tuberculous foci. These lesions are simply the accumulation of megacaryocytes that have emigrated from the bone marrow to the areas of damaged tissue. They are not true Hodgkin's lesions. The participation of the megacaryocyte in an acute tuberculous process is emphasized. This is not specific for tuberculosis since it occurs in other pathologic conditions. The necessity of exercising care in the diagnosing of Hodgkin's disease from tissue sections is stressed. The presence of the Sternberg type of giant cell in tissue is not sufficient evidence to warrant a diagnosis of Hodgkin's disease. Various types of virulent tubercle bacilli cause the production of the lesions that simulate Hodgkin's disease. This would seem to preclude the possibility that any one type of tubercle bacillus is the etiologic agent of Hodgkin's disease. Lesions suggestive of Hodgkin's disease have been observed only in acute tuberculosis. In chronic tuberculosis these lesions have not been encountered.

AUTHORS' SUMMARY.

THE DENTAL ANLAGE IN CONGENITAL SYPHILIS. T. J. HILL, *Am. J. Path.* 7:515, 1931.

The characteristic congenital syphilitic dental deformities are not the direct result of the invasion of the enamel organ by *Treponema pallidum*.

AUTHOR'S SUMMARY.

INFECTION OF MONKEYS WITH GRANULOMA INGUINALE. W. A. DEMONBREUN and E. W. GOODPASTURE, *Am. J. Trop. Med.* 11:311, 1931.

Three *Macacus rhesus* monkeys inoculated with Donovan organisms from a case of granuloma inguinale showed evidence of an abortive infection with this agent. A biologic method is thus afforded for testing any micro-organism that might be cultivated from these lesions and suspected of being the etiologic agent.

AUTHORS' SUMMARY.

THE PATHOGENIC ACTION OF FILTRATES OF CULTURES OF TUBERCLE BACILLI IN GUINEA PIGS. MAX PINNER and M. VOLDRICH, *Am. Rev. Tuberc.* 24:73, 1931.

Filtrable forms of the tubercle bacillus are not demonstrable in pure cultures of tubercle bacilli under the stated conditions; their existence has not been proved in a convincing manner. Broth-culture filtrates from tubercle bacilli cause a characteristic disease in guinea-pigs (marked by rapid loss of weight, dehydration and loss of tissue tone without local lesions). The nosogenic agent of this disease is not a living virus, because (1) it is not present in whole cultures of tubercle



bacilli, grown on solid mediums; (2) it is thermostabile, and (3) it is not transmissible by animal passage. This disease is probably caused by a specific tuberculotoxic substance that may be identical with tuberculin.

AUTHORS' CONCLUSIONS.

TUBERCULOSIS OF THE STOMACH. R. W. GOOD, Arch. Surg. **22**:415, 1931.

The genesis of tuberculosis of the stomach is not settled. Ulcer is the common lesion. Abscesses may form and burrow through the wall. Regional lymph nodes are usually involved. The coincidence of gastric carcinoma and tuberculosis is rare. About twenty-six cases have been collected from the literature. Over half of the cases are associated with tuberculosis elsewhere in the body. It is usually mistaken for carcinoma.

N. ENZER.

THE DERIVATION OF ENCAPSULATED FORMS FROM NON-ENCAPSULATED HEMOLYTIC STREPTOCOCCI. F. B. JENNINGS, JR., Bull. Johns Hopkins Hosp. **49**: 94, 1931.

Seven strains of nonencapsulated hemolytic streptococci, including erysipelas and scarlet fever strains, have been dissociated, in vitro, to encapsulated forms. It seems probable that the potentiality to form capsules may be a characteristic of hemolytic streptococci in general. Dissociation to the encapsulated form enhances the culture's virulence for mice to a high degree. The encapsulated forms have retained their morphology and virulence, without animal passage (in vitro) since their dissociation—a period which has been from one to three years.

AUTHOR'S SUMMARY.

THE RAT FLEA AS VECTOR OF TYPHUS FEVER. H. A. KEMP, J. A. M. A. **97**: 775, 1931.

Guinea-pigs inoculated with fleas removed from rats that had been trapped at a typhus focus developed lesions characteristic of endemic typhus fever. Animals recovered from an attack produced by this virus were found to be immune to a strain of typhus virus established from the blood of a human patient with endemic typhus. Animals that were immune to blood virus were immune to the strain of rat flea virus established by guinea-pig inoculation.

AUTHOR'S SUMMARY.

CULTIVATION OF RICKETTSIA-LIKE MICROORGANISMS FROM CERTAIN BLOOD-SUCKING PUPIPARA. I. J. KLIGLER and M. ASCHNER, J. Bact. **22**:103, 1931.

Experiments are reported dealing with the cultivation of extracellular non-pathogenic rickettsias from a number of pupipara. Methods are described by means of which cultures of Rickettsia were obtained repeatedly from the parasitic pupipara of the sheep, goat, horse and dog. The organisms obtained in culture were minute, gram-negative, coccoid rods corresponding to the usual description of Rickettsia and resembling the forms seen in the intestines of the insects.

AUTHORS' SUMMARY.

THE RECOVERY OF BACTERIOPHAGE FROM FILTRATES OF HEATED SPORE-SUSPENSIONS. P. B. COWLES, J. Bact. **22**:119, 1931.

That bacteriophage may be demonstrated in growth resulting from spores that have been heated to a much higher temperature than that tolerated by free bacteriophage was shown by den Dooren de Jong, who on this basis concluded that the lytic principle was generated in the cell. In this paper evidence is presented to show that known bacteriophage-free strains of several spore-forming

organisms may be so changed by the action of the bacteriophage that the principle can be demonstrated in filtrates of cultures developed from heated spores. This does not necessarily mean that the lytic agent as such survives the heating process, but it does mean that the recovery of a lytic principle from a pasteurized culture is not conclusive proof of the spontaneous generation of bacteriophage.

## AUTHOR'S SUMMARY.

THE BEHAVIOR OF ACID-FAST BACTERIA IN OIL AND WATER SYSTEMS. B. G. REED and C. E. RICE, *J. Bact.* **22**:239, 1931.

On shaking watery suspensions of acid-fast bacteria with oil, they readily pass into the oil, while nonacid-fast bacteria remain in the water. The same reaction occurs with a variety of fat solvents. The partition into oil is shown quantitatively to be nearly complete for tubercle bacilli and from 70 to 90 per cent for six other acid-fast species. Homologous immune serum inhibits, in proportion to its concentration, the partition from water to oil. Emulsifying agents such as gelatin and sodium oleate have a similar effect.

## AUTHORS' SUMMARY.

THE ELECTRICAL CHARGE OF BACTERIOPHAGE. C. E. CLIFTON and R. R. MADISON, *J. Bact.* **22**:255, 1931.

The electrical charge carried by the bacteriophage was determined by noting the tendency of this agent to climb with water in blotting paper. The results presented herein indicate that the iso-electric point of the anticoli bacteriophage tested is around  $p_H$  2.9; of antishiga bacteriophage, near  $p_H$  3.1; of antistaphylococcus bacteriophage, approximately at  $p_H$  3.5, and of antipyocaneus bacteriophage, below a  $p_H$  of 2.1.

## AUTHORS' SUMMARY.

TULAREMIA IN NORWAY. TH. THJØTTA, *J. Infect. Dis.* **49**:99, 1931.

Tularemia is not a rare disease in Norway. It has been found over nearly the whole country. With the exception of the typhoidal type of the disease, all other types have been encountered. No deaths have been seen. Besides the hare, the lemming is probably a carrier. No case originating from the bite of an insect has been seen as yet.

## AUTHOR'S SUMMARY.

BRUCELLA ABORTUS. W. N. PLASTRIDGE and J. G. McALPINE, *J. Infect. Dis.* **49**:127, 1931.

One hundred and thirty-six strains of *Br. abortus* of bovine, porcine and equine origin were identified by the dextrose utilization method of McAlpine and Slanetz, and by Huddleson's dye plate method. Eight of sixty strains of bovine origin isolated in the United States were found to be of the porcine, and fifty-two of the bovine, type. Of fifty strains of bovine origin isolated in Europe, forty-eight were found to be of the bovine type, while two appeared to be of intermediate types. All of the twenty-two strains of *Br. abortus* of porcine origin utilized appreciable amounts of dextrose and behaved as the porcine type on the dye plates. Four strains of equine origin proved to be of the bovine type of *Br. abortus*.

## AUTHORS' SUMMARY.

THE HERROLD EGG YOLK AGAR MEDIUM IN THE DIAGNOSIS OF TUBERCULOSIS. C. I. WOOLSEY, *J. Infect. Dis.* **49**:177, 1931.

One egg yolk added to 200 cc. of 1.5 per cent agar at 60 C., mixed well, makes a valuable medium. The results of cultures on this medium (Herrold) and of inoculations into guinea-pigs agreed in 93 per cent of 130 cases; in 5 of the 9 cases in which the results disagree, the Herrold cultures were found the more reliable. Thirty-six strains of tubercle bacilli were recovered on the Herrold

medium in an average of 12.2 days. Mixing freshly aspirated fluids from tuberculous processes with an equal amount of 20 per cent sodium citrate solution prevents coagulation. However, the entire coagulum may be placed on the Herrold medium and growths of tubercle bacilli obtained in that way.

AUTHOR'S SUMMARY.

HERPETIC VIRUS IN MICE. H. B. ANDERVONT, *J. Infect. Dis.* **49**:507, 1931.

Continued passage of the herpetic virus through the brains of mice did not increase its pathogenicity for rabbits. Male mice appear to be more susceptible to cutaneous inoculation of the JB virus, and there is also evidence of seasonal variation in the susceptibility of the mice. Nitric acid and formaldehyde destroy the JB virus when applied to the infected skin. Practically all mice showing a local reaction after injection of the JB virus into the abdominal skin develop encephalitis, but many survive after cutaneous inoculation of the tail. These tail-inoculated mice are immune after three days to further cutaneous or intracranial inoculation of JB virus. Mice can also be immunized by intraperitoneal injections of formaldehydized suspensions of brain infected with JB virus.

FROM AUTHOR'S SUMMARY.

INTERSTITIAL PERIPHERAL NEURITIS IN EXPERIMENTAL POLIOMYELITIS. A. JORDI, *J. Infect. Dis.* **49**:530, 1931.

The peripheral nerves in seventeen of twenty monkeys that were infected experimentally by intracerebral and intranasal methods showed a definite interstitial inflammation. The perivascular infiltrations, mostly by round cells, were generally small and inconspicuous and were not numerous. Infiltrations were found as early as twelve hours after the onset of paralysis. However, in the three animals the nerves of which were free from infiltrations and in a few animals in which not all the extremities were affected, paralysis had existed for less than six days. Infiltrations were present in the nerves of the extremities that were not paralyzed. The sciatic nerves of one normal monkey which were partly cut, respectively, twelve and four days before the animal was killed showed secondary degeneration, but no signs of interstitial inflammation, thus proving that the interstitial neuritis in experimental poliomyelitis is caused by the specific virus. In early stages of poliomyelitis, necrosis of the ganglion cells, both in the spinal cord and in the intervertebral ganglion cells, and not the interstitial inflammation, is the first manifestation of the virus.

AUTHOR'S SUMMARY.

RELATION BETWEEN BACTERIA AND TEMPERATURE IN SUBACUTE BACTERIAL ENDOCARDITIS. H. WEISS and R. OTTENBERG, *J. Infect. Dis.* **50**:61, 1932.

The relationship of the temperature to the bacteremia in four cases of subacute bacterial endocarditis indicates that a correlation between these two phenomena probably exists. The bacteremia is apparently maintained (1) by a constant, practically uniform feeding of bacteria into the blood stream from the endocardial vegetations and (2) by the sudden liberation of a large number of organisms. The bacteremia in each case apparently establishes a balance between bacterial invasion and immunologic response, which it maintains throughout the course of the disease. The clinical severity of the disease bears no relationship to the degree of bacteremia. The results indicate that if diagnostic blood cultures, usually made at the time of hyperpyrexia, are made just before the expected rise in temperature, more positive results and higher bacterial counts will be obtained.

AUTHORS' SUMMARY.

DIPHATHEROIDS IN BLOOD CULTURES. L. THOMPSON, *J. Infect. Dis.* **50**:69, 1932.

Of 1,079 blood cultures, 33 (3 per cent) gave a growth of diphtheroids. Study of 17 strains disclosed great variation among the organisms in regard to morpho-

logic and cultural characteristics. It was not possible to correlate the finding of diphtheroids in the blood cultures with the clinical diagnosis. In none of the cultures studied was any marked change from the morphology of the diphtheroids noted. An instance is cited in which a pleomorphic streptococcus was obtained from the blood in a case of subacute bacterial endocarditis. Diphtheroids appear to have the same significance in blood cultures as the saprophytic cocci.

AUTHOR'S SUMMARY.

CHARACTERISTICS OF *MONILIA* FROM HUMAN SOURCES. W. D. STOVALL and A. A. BUBOLZ, *J. Infect. Dis.* **50**:73, 1932.

One hundred and fifty cultures of *Monilia* isolated from cases of bronchitis, vaginitis and thrush and ten secured from the American Type Culture Collection were studied. By morphology of colonies, fermentation of carbohydrates and behavior toward milk all cultures were classified into three species. Two species were represented in type cultures, *M. albicans*, type II, and *M. candida*, type III. One species, Type I, was rarely found associated with human disease. *M. albicans* was isolated more frequently than *M. candida*. The authors conclude that many cultures of *Monilia* described as different species really belong to these two types. The cultural and biochemical characteristics were remarkably constant, and the reported variability is thought to be due to a lack of uniformity in technic.

EDNA DELVES.

METABOLIC STUDIES OF STREPTOCOCCI. M. A. FARRELL and S. THOMAS, *J. Infect. Dis.* **50**:134, 1932.

Nitrogen supplied in the form of ammonium salts, asparagine and uric acid failed to stimulate or support the growth of *S. rheumaticus*. When rhamnose, levulose or galactose was added to a nonprotein source of nitrogen, growth was secured to the third transplant. When the nonprotein nitrogen was enriched with an additional source of nitrogen in the form of an amino-acid, no growth was obtained. The original inoculum survived in some of the added amino-acids up to forty-eight days, while other amino-acids were antagonistic, the bacteria being destroyed at the end of two days. When any one of the various sugars was added to the amino-acid that was the least inhibitory, no growth resulted. When dextrose was added to each of the nine amino-acids, synthetic mediums being used as substrates, the antagonistic action of tyrosine, tryptophan and phenylalanine was shown to be lessened. *S. rheumaticus* grew well on milk aminoids.

AUTHORS' SUMMARY.

VARIANTS OF *SALMONELLA PULLORUM*. W. PLASTRIDGE and L. F. RETTGER, *J. Infect. Dis.* **50**:146, 1932.

Extracts of the livers and the intestinal contents of adult birds surviving an acute attack of disease due to infection with *S. pullorum* were found to contain a bacteriophage that was active against the cells of *S. pullorum* in dilutions including  $1 \times 10^{-10}$ . In the presence of this active principle, cells of *S. pullorum* in most instances were lysed or became phage-resistant, and in the other instances the development of the cells appeared to parallel the action of the bacteriophage. Under the last-named condition, cells of *S. pullorum* were inagglutinable by antiserum for the ordinary *S. pullorum*. Three principal types of colonies were observed on beef infusion and liver infusion agars inoculated with the mixtures of phage and culture; namely, S1 or ordinary smooth colonies of *S. pullorum*, S2 colonies that were smooth in appearance, but much larger than S1 colonies, and R colonies that possessed distinctly roughened surfaces. Colonies developing on ordinary nutrient agar inoculated with cells from R colonies showed only a slight tendency toward roughness. The morphology, agglutinability and colonial characteristics of sub-strains of these three types of colonies are discussed.

AUTHORS' SUMMARY.

INTRANUCLEAR INCLUSIONS IN THE SUBMAXILLARY GLAND OF THE RAT. M. J. THOMPSON, J. Infect. Dis. **50**:162, 1932.

Specific inclusions were found within the nuclei of cells in the ducts of the submaxillary gland of the rat. Fourteen per cent of a series of seventy 2 months old rats showed these affected cells. The incidence was suggestive of either a seasonal variation or of an epizootic. The histologic changes in the submaxillary gland of the guinea-pig infected with submaxillary virus have been compared with those in the affected gland of the rat. It is believed that one is dealing with a disease caused by a virus different from that causing the submaxillary condition of the guinea-pig. The submaxillary lesion in the rat possesses features in common with the lesions of other diseases due to viruses.

AUTHOR'S SUMMARY.

CONCERNING THE DISTRIBUTION OF DIPHYLLOBOTHRIUM LATUM IN NORTH AMERICA. T. B. MAGATH and H. E. ESSEX, J. Prev. Med. **5**:227, 1931.

Fish were examined from practically every watershed in Minnesota. Infested fish were found in four lakes, three of which drain by northern passage into Canadian lakes; the fourth drains into Lake Superior. The fish found infested were of the following kinds: *Esox lucius*, *Stizostedion vitreum* and *Perca flavescens*. The following plankton forms were found to be first intermediate hosts for the parasite: *Diaptomus oregonensis* (previously reported by Essex), *Diaptomus sicilis* and *Diaptomus siciloides*. The primary introduction of *Diphyllobothrium latum* into the waters of North America was made by immigrants accustomed to eat raw fish, who came from the endemic centers of Europe, in particular from the Baltic states, northern Sweden, Denmark, Finland, Lithuania and Germany. Many of these people, particularly the Scandinavians and Finlanders, have settled in the lake regions of central North America, where the smaller lakes contain suitable intermediate hosts to insure the development of the worm. The temperatures at the bottom of the lakes play a part in determining whether the eggs will hatch. In many of the cities and towns of Minnesota and Canada where these infested persons have congregated, raw sewage is emptied into the lakes or streams, and in other towns the sewage systems will not prevent the escape of eggs or coracidia. Moreover, chance contamination of these lakes by man is probably very common, since the people are ardent hunters, fishermen and trappers, as well as lumbermen. The high viability of eggs from human sources increases the hazard. Water connections between the lakes allow fish to migrate and carry infestation to new streams and lakes. From the several fish hatcheries situated in these infested regions fish are transported and planted in many waterways. If the hatchery water is taken from a lake contaminated with *Diphyllobothrium latum* eggs, the chances are great that the susceptible fish will become infested and carry the infestation when transported to some far removed lake. That animals other than man will carry infestation to the fish of uninfested waters and that the infestation will maintain itself in the new lake is a possibility as yet unproved. No one has positively recorded finding the worm in nature in any wild animal in North America except in the case of one fox. Dogs are readily infested, and many of those in the lake regions harbor the worm, but their part in the spread of the infestation seems at present more hypothetical than practical. In the light of present knowledge the control of infestations by *Diphyllobothrium latum* lies chiefly in the hands of man.

AUTHORS' SUMMARY.

THE ADSORPTION AND ELUTION OF BACTERIOPHAGE AND FOWL-POX VIRUS. I. J. KLIGLER and L. OLITZKI, Brit. J. Exper. Path. **12**:172, 1931.

*Coli* bacteriophage and the virus of fowl-pox are completely adsorbed by kaolin and can be partially recovered from the adsorbent by elution with dilute ammonium hydroxide. Chemical tests for proteins in the purified suspension are negative.

C. E. CLIFTON.

### Immunology

HUMAN BLOOD IN PROTECTION AGAINST MUMPS. LOUIS H. BARENBERG and JACK OSTROFF, *Am. J. Dis. Child.* **42**:1109, 1931.

A study was made of 180 children, ranging in age from 1½ to 3 years, and housed in nine separate wards, who were susceptible to mumps. Serotherapy was employed in 55 of these 180 susceptible children in order to reduce the incidence or to attenuate the disease. (At the onset of the epidemic, 44 children received 12 cc. of blood from convalescent patients, and 11 children the same amount of blood from an adult who had had mumps in childhood.) In seven wards, comprising 139 children, prophylactic treatment was given to about half the children in each ward, the rest serving as controls. Those in two other wards were not given specific therapy, and consequently afforded an excellent opportunity for comparison.

The average incidence in the untreated wards was 49.5 per cent, whereas in the other seven wards, in which treatment was given to some of the children, the average incidence was 26.6 per cent. Serotherapy proved of definite value in reducing the incidence of mumps, the average for the treated children being 15 per cent, and that for the untreated children, 39 per cent. Mumps was markedly attenuated by this form of serotherapy.

The results from the injection of blood from an adult who had had mumps in childhood were not as definitely favorable as those from the injection of blood of patients convalescent from mumps, perhaps because inadequate amounts were injected.

A definite shortening of the epidemic was brought about by the use of serotherapy; from a period of about six months in previous epidemics the period dropped to about two months in the present one.

AUTHORS' SUMMARY.

HYPERSENSITIVITY TO BACTERIAL PROTEINS AND ITS RÔLE IN SUSCEPTIBILITY AND IMMUNITY. W. B. WHERRY, *Am. J. Hyg.* **14**:539, 1931.

Bacteria are potential parasites if they can produce poisons that hydrate the tissues of an animal, thus putting the food substances into solution. Some of the poisons produced might be amines. The fact that bacteria contain poisons capable of hydrating tissues may be determined by the intradermal inoculation of the bacteria, extracts of the bacteria or their toxins, or by immersing tissues in the toxins in the presence of free water. A state of hypersensitivity to bacterial toxins, extracts or heat-killed bacteria may be determined by the intradermal test. Such tests have shown that persons suffering from many infections, including asthma, acute and chronic sinusitis, colds, urticaria, angioneurotic edema, spastic and mucous colitis and chronic arthritis, are hypersensitive to bacterial proteins. These cases can be relieved by desensitization, and this is best brought about by partially detoxicated antigens. Amines and foreign proteins pass the mucosa best in an alkaline medium. Cases showing intoxication or sensitization to proteins derived from the colon show evidences of putrefaction taking place in an alkaline medium. The saccharolytic colonic flora is suppressed in these cases, and the symptoms may be relieved by feeding lactose. Keeping the contents of the colon in an acid state should guard against the development of such types of hypersensitivity.

AUTHOR'S SUMMARY.

THE PRESENCE OF HETEROPHILE ANTIBODIES IN INFECTIOUS MONONUCLEOSIS. JOHN R. PAUL and W. W. BUNNELL, *Am. J. M. Sc.* **183**:90, 1932.

Heterophile antibodies, demonstrable in the form of sheep cells agglutinins have been recorded in rather high concentrations in the active stages of four cases of infectious mononucleosis. Apart from cases of serum disease, and one notable exception, we have failed to note this in a large series of cases representing a variety of clinical conditions, including Vincent's angina, lymphatic leukemia and

other blood dyscrasias. There would seem to be two possible explanations for this finding: (1) that the unknown agent responsible for infectious mononucleosis contains the heterophile antigen; (2) that we are dealing with an example of the production of iso-agglutinins elicited by abnormal cells, which are present either in the blood or elsewhere during active stages of the disease.

AUTHORS' SUMMARY.

THE NATURE OF ALLERGY IN TUBERCULOSIS AS REVEALED BY TISSUE CULTURE STUDIES. A. R. RICH and M. R. LEWIS, Bull. Johns Hopkins Hosp. 50:115, 1932.

The washed cells of the allergic, tuberculous body have been shown to retain their hypersensitivity to tuberculin when isolated from the body in tissue cultures. It is therefore clear that circulatory, nervous or other mechanisms dependent on the intact body are not necessary for the production of allergic damage. Cellular injury and necrosis associated with allergy in tuberculosis result from a change in the individual fixed tissue and blood cells, which renders them hypersensitive to the protein of the tubercle bacillus. This change, in all probability, consists in the intimate attachment of antibody to the cells. Free, circulating antibody is not necessary for the production of the immediate, local injurious effects of allergy in tuberculosis.

AUTHORS' SUMMARY.

THE AGGLUTINATION OF BACILLUS SORDELLII AND CLOSTRIDIUM OEDEMATOIDES. I. C. HALL and A. L. SCOTT, J. Bact. 22:375, 1931.

There is cross-agglutination between all strains of *B. sordellii* and *C. oedematoides*. Serum prepared against *B. sordellii* or against *C. oedematoides* does not cross-agglutinate *B. sporogenes*, *B. tyrosinogenes* or *B. novyi*. *B. sordellii* and *C. oedematoides* are therefore distinct serologically from other species of obligately anaerobic bacilli.

AUTHORS' SUMMARY.

SYPHILITIC BLOOD PROTEINS (SURFACE TENSION AND SOLUBILITY). S. T. WALTON, J. Exper. Med. 54:859, 1931.

The surface tension of normal blood serum is considerably lowered by standing undisturbed for a period of one hour (time drop). The greatest time drop recorded is with serum diluted approximately 10,000 times in fresh serum or 50,000 times in heated serum. Immune serum is not affected in the same manner by heat as is normal serum. Syphilitic serum and antisherp cell rabbit serum behave similarly in this respect. The albumin of serum is much more readily soluble in alkaline buffer solutions than the globulin is, and globulin from normal serum ionizes more than that from syphilitic serum. Further investigations are being made in an effort to determine why the proteins aggregate or dissociate under the influence of the factors under consideration.

AUTHOR'S SUMMARY.

ORAL IMMUNIZATION AGAINST PNEUMOCOCCUS TYPES II AND III, AND TYPE I. V. ROSS, J. Exper. Med. 54:875 and 899, 1931.

Older rats survive greater doses of *Pneumococcus* type II than young ones, showing the acquisition of a partial natural immunity. The same is true with regard to type III, but the immunity appears later in life. An active immunity can be created against types II and III in rats by feeding dead organisms or the Berkefeld filtrate of the bile salt-dissolved cells. This immunity resembles that obtained against type I. Feeding the purified soluble specific substance of type I protects rats against an intraperitoneal injection of the virulent organism. This increased resistance in some ways resembles that obtained when the dead or dissolved bacteria are fed, but the proportion of animals protected and the height of the immunity are not as great. Mice were not protected by immunization with the purified soluble specific substance. A sodium glycocholate solution of type I lost part of its immunizing power after standing one year.

EDNA DELVES.

ORAL IMMUNIZATION WITH SOLUBLE SPECIFIC PNEUMOCOCCAL SUBSTANCE. V. ROSS, *J. Exper. Med.* **55**:1 and 13, 1932.

When the soluble specific substance of *Pneumococcus* type II was fed to rats, little or no increased resistance to the organism was obtained. When the specific substance of type III was fed, an increased resistance to the virulent organism resulted. The percentage of animals protected is smaller than when the whole or the dissolved cell is fed. When the specific polysaccharide of type I was fed, no immunity against type II or type III was obtained, and the ingestion of the specific substances of types II and III did not protect rats against type I.

Rats, when fed the soluble specific substances of types I, II and III, excrete a very large proportion in the feces. Following ingestion of these substances, tests sensitive enough to detect 1 part in 2,000,000 of serum, and 1 part in 3,000,000 of urine, were negative. The polysaccharide of type I, when recovered from the feces, is as active an immunizing agent as it was originally.

AUTHOR'S SUMMARY.

THE IDENTITY OF TYPE-SPECIFIC AGGLUTININ AND PRECIPITIN REACTIONS WITH PNEUMOCOCCUS. T. FRANCIS, JR., *J. Exper. Med.* **55**:55, 1932.

The results obtained in the agglutination reaction conform qualitatively with the principles described by Heidelberger and Kendall for the phenomenon of precipitation. They indicate that the essential mechanisms of the two reactions are identical, and that the active reagents are the same in both.

AUTHOR'S SUMMARY.

PRECIPITINOGEN AND PRECIPITINS IN RELATION TO SERUM SICKNESS IN RABBITS. L. JONES and M. S. FLEISHER, *J. Exper. Med.* **55**:79, 1932.

It has not been possible to demonstrate in rabbits affected with serum sickness any constant temporal relationship between precipitin and precipitinogen in the blood, on one hand, and occurrence of serum sickness, on the other hand. It has not been possible to demonstrate any differences between the precipitin and precipitinogen curves of rabbits in which serum sickness developed and those of rabbits in which it did not develop. There is, therefore, no evidence that the occurrence of serum sickness can be directly associated with the production or the appearance of precipitins.

AUTHORS' SUMMARY.

THE DISAPPEARANCE OF LIVING BACTERIA FROM THE BLOOD STREAM. P. R. CANNON, F. L. SULLIVAN and E. F. NECKERMANN, *J. Exper. Med.* **55**:121, 1932.

The simultaneous intravenous injection into normal and actively immunized rabbits of equal quantities of living staphylococci or paratyphoid bacilli is followed by a distinctly accelerated rate of removal of the bacteria from the blood streams of the immune animals. This altered reactivity is due essentially to specific active immunization. The bacteria pass rapidly through the capillary bed of the lungs, extracellularly and dispersed for the most part, and become generalized through the blood stream. The bacteria are quickly removed from the circulating blood in the immune animals and less rapidly in the normal ones, by various organs, particularly the liver and spleen, where they accumulate in enormous numbers, become adherent to the lining membrane of the sinusoides of the liver and apparently to the macrophages of the spleen and are phagocytosed by the macrophages and leukocytes in these organs. Associated with this effect are morphologic changes in the bacteria, as shown by swelling, loss of staining power and evidences of increased cohesiveness and decreased viscosity, these changes being apparent as early as two minutes after their intravenous injection. As these changes are not seen to a marked degree within the lungs or other organs, they are probably the result of a local antigen-antibody reaction of a bacteriotropic type in the two



organs generally considered to be most actively concerned with the production of immune bodies. By means of this accelerated bacteriotropic effect in the actively immunized animals, phagocytosis is facilitated and intracellular digestion of the bacteria is enhanced.

AUTHORS' SUMMARY.

LOCAL ANAPHYLACTIC INFLAMMATION IN THE RABBIT PERICARDIUM, HEART, AND AORTA. D. SEEGAL, B. C. SEEGAL and E. L. JOST, J. Exper. Med. **55**:155, 1932.

An intense inflammatory reaction in the pericardium, heart and intrapericardial aorta can regularly be produced in a sensitized rabbit by the intrapericardial injection of the homologous antigen.

AUTHORS' SUMMARY.

LOCAL ANAPHYLACTIC INFLAMMATION IN THE RABBIT BRAIN. L. M. DAVIDOFF, B. C. SEEGAL and D. SEEGAL, J. Exper. Med. **55**:163, 1932.

Sixteen of seventeen rabbits actively sensitized to various antigens by repeated cerebral and intravenous injections showed on intracerebral reinjection of the same antigen local anaphylactic inflammation of the brain at the site of inoculation. Six of twenty rabbits actively sensitized to either horse serum or egg albumin by extracerebral injections showed, on introduction of the homologous antigen into the cerebrum, local anaphylactic inflammation at the site of inoculation. The pathologic picture of Arthus' phenomenon in the brain of the rabbit resembled that seen in the skin, after allowing for differences in the fundamental structure of the tissues involved. None of the control animals exhibited lesions comparable to those found in the experimental animals. A few controls showed slight hemorrhages due to mechanical injury of blood vessels. Clinical symptoms of varying degrees of severity, often leading to the death of rabbit, were observed in the sensitized animals. These symptoms were referable to the site of injection.

AUTHORS' SUMMARY.

IMMUNOLOGICAL REACTIONS OF PNEUMONIC PLEURAL FLUIDS. M. FINLAND, J. Exper. Med. **55**:169, 1932.

Pleuritic exudates from patients with lobar pneumonia may be sterile or infected. Sterile fluids, at or about the time of crisis, contain actively acquired antibodies similar to those in the blood serum. Infected fluids do not contain such antibodies, presumably because of the presence in them of large amounts of soluble specific substance. Sterile fluids from patients treated with immune serum contain both horse serum and antibodies similar to those injected. Infected fluids from serum-treated persons contain horse serum and such heterologous antibodies as were contained in the therapeutic serum together with homologous soluble specific substance. The concentrations of horse serum and antibodies in pneumonic fluids are usually the same or somewhat less than those in corresponding blood serums.

AUTHOR'S SUMMARY.

TOXIN-ANTITOXIN REACTION WITHOUT NEUTRALIZATION. J. FREUND, J. Exper. Med. **55**:181, 1932.

Collodion particles adsorb diphtheria, tetanus and botulinus toxins. These toxins are retained on the particles when washed, but are at least in part released in the animal. The adsorbed toxins are neutralized by adsorption of the corresponding antitoxins, but are unaffected by other serums. When collodion particles are treated first with tetanus antitoxin, then with diphtheria toxin, they are not toxic, but they become toxic when they are treated first with diphtheria antitoxin, then with diphtheria toxin. Similarly when collodion particles are treated first with diphtheria antitoxin and then with tetanus toxin, they do not become toxic, but they become toxic when they are treated with tetanus antitoxin and tetanus toxin.

AUTHOR'S SUMMARY.

SPECIFIC AGGLUTINATION OF PNEUMOCOCCUS TYPES I, II AND III. J. F. ENDERS, J. Exper. Med. **55**:191, 1932.

Type-specific agglutination of *Pneumococcus* types I, II and III has been demonstrated in antisera largely deprived of the carbohydrate antibody. The type-specific agglutinogens in *Pneumococcus* I and II responsible for agglutination in such antisera are inactivated by heating in alkaline solution at 100 C. The specific carbohydrate remains unaltered under these conditions. The relationship of the type-specific agglutinin in *Pneumococcus* I to the type-specific A substance has been discussed. The possible application of these results to the standardization of therapeutic antisera by agglutinin titer has been discussed. On the basis of the experiments recorded, a tentative explanation is offered for the failure of *Pneumococcus* type II to absorb the agglutinins from serum prepared against Friedländer's bacillus type B.

AUTHOR'S SUMMARY.

ANTISERUM IN INFLUENZAL MENINGITIS. H. K. WARD and J. WRIGHT, J. Exper. Med. **55**:223, 235, 1932.

Acute purulent meningitis due to invasion of the meninges by Pfeiffer's influenza bacillus is not uncommon in infants and children. The mortality is very high. Complement is entirely lacking in the cerebrospinal fluid in these cases, and bactericidal experiments suggest that the injection of a specific antiserum has but slight lethal effect on the organisms unless complement is injected at the same time. Treatment with a mixture of specific antiserum and complement led in some cases to a definite clinical improvement, coincident with sterilization and clearing of the spinal fluid. But after some days, the patient had a relapse and died. Autopsy showed localized abscesses in the vicinity of the base of the brain, the lesions being definitely walled off from the subarachnoid space. In one case the patient recovered. Since the walls of the abscesses apparently present an insuperable mechanical obstacle to the action of the antiserum and complement, the possibility of preventing the formation of abscesses is discussed. Earlier diagnosis and more rapid sterilization are the most obvious measures. Bactericidal experiments indicate that the proportion of antiserum to complement may be an important factor in bringing about a more rapid elimination of the bacilli.

By a suitable bactericidal technic, it can be demonstrated that the virulent *S* influenza bacillus is completely resistant to the bactericidal action of diluted normal unheated serum. In contrast, the *R* organism is easily killed when subjected to diluted normal serum. Although this is not a true test of virulence, it promises to be a useful substitute when a susceptible animal is not available. The filtrate of the *S* culture contains a substance with a strong antibactericidal effect, but that of the *R* culture does not contain this substance. It would appear probable that this antibactericidal substance is identical with, or closely related to, the precipitinogen (or soluble substance), which is present only in the culture fluid of the *S* influenza bacillus. In view of differences in the heat stability of the two substances, this question must remain in doubt until a supply of purified precipitinogen is available. Comparisons of quantitative agglutination, precipitation and bactericidal action between (a) *R*-absorbed anti-*S* serum and unabsorbed anti-*S* serum and (b) anti-*S* serum and anti-*R* serum indicate that the agglutinin is a separate antigen, and the agglutinin a separate antibody, taking no part in the bactericidal action of the antiserum. They also indicate that the precipitin, which is present only in the anti-*S* serum, is identical with the bactericidal antibody.

AUTHORS' SUMMARIES.

ANTIGENIC STREPTOCOCCAL HEMOLYSIN. E. W. TODD, J. Exper. Med. **55**:267, 1932.

Normal serum used in cultures for preparation of streptococcal hemolysin modifies the properties of the streptococcal hemolysin, causing delayed hemolysis, increased filtrability, resistance to oxidation or reduction and absence of antigenicity. Streptococcal

prepared without serum is an active antigen. Similar temperatures are required to destroy the antigenic activity of serum-free streptocolysin and the intracutaneous reactivity of Dick toxin. Scarlet fever antitoxin contains antistreptocolysin which does not neutralize serum streptocolysin and which can be detected only by titration against serum-free streptocolysin. The antihemolysin which neutralizes serum-free streptocolysin is species-specific but not type-specific.

AUTHOR'S SUMMARY.

THE CORRELATION BETWEEN PROTECTIVE VALUE AND OTHER IMMUNOLOGIC REACTIONS OF TYPE I ANTIPNEUMOCOCCUS SERUM. L. D. FELTON, J. Immunol. **21**:341, 1931.

Thirty-nine freshly drawn polyvalent type I and type II antipneumococcus horse serums were studied to find the degree of correlation between the protective titer and various immunologic reactions (specific precipitation, agglutination and neutralization) and immune protein precipitable by soluble carbohydrate. It has been found that for type I the correlation coefficient between protection and precipitin titer is 0.93; between protection and agglutination, 0.80; between protection and neutralization, 0.88, and between protection and the amount of protein precipitated with specific carbohydrate, 0.91. From this degree of correlation it appears evident that, at least for type I freshly drawn serums, immunologic examination other than the expensive mouse protection test can be utilized to estimate the probable therapeutic activity and to standardize antipneumococcus serum. Reproducible and accurate results in my experience have been possible by the use of the amount of protein in the serum precipitable with the soluble specific carbohydrate.

AUTHOR'S SUMMARY.

ETHYL ALCOHOL IN THE CONCENTRATION OF ANTIPNEUMOCOCCUS SERUM. L. D. FELTON, J. Immunol. **21**:357, 1931.

Studies of the solubility of the pneumococcus antibody in alcohol reveal the following: Practically all the protective substance present in the original serum is insoluble in 15 to 20 per cent alcohol at 0 C. The precipitated protein containing both water-soluble and water-insoluble protein can be washed with water to remove the former, and the latter dissolved in salt solution is found to contain protective antibody in practically the same concentration as the original serum. Precipitation with 10 per cent alcohol gives a product in which the immune protein is approximately 80 per cent of the total protein. Irrespective of hydrogen ion concentration from  $p_H$  2 to  $p_H$  9, if the precipitate is neutralized before diluting, the pneumococcus antibody is insoluble in 20 per cent alcohol. In more alkaline solutions, a greater concentration of alcohol is necessary to cause precipitation. Water-alcohol mixtures precipitate the antibody but with an increased amount of inert protein as compared with the precipitate obtained with alcohol mixed with undiluted serum. The use of alcohol as a precipitant in the concentration of antipneumococcus serum gives a yield of at least 80 per cent of the protective substance found in the original serum. It has been found possible to dry the active material with retention of immunologic characteristics.

AUTHOR'S SUMMARY.

FLOCCULATION OF VACCINIAL VIRUS-TISSUE SUSPENSIONS BY SPECIFIC ANTISERUM. R. THOMPSON and L. BUCHBINDER, J. Immunol. **21**:375, 1931.

The flocculation of suspensions of skin containing vaccinia virus by homologous antiserum noted by Gordon and utilized by Burgess et al. in the diagnosis of variola has been readily confirmed. The same antigens were also flocculated by serums prepared against brain and testicle containing vaccinia virus, but not by serums from rabbits immunized against normal skin, normal brain, normal testicle or streptococcal skin. The serums from rabbits that had recovered from extensive vaccinia infections of the skin did not flocculate the suspensions of virus-containing skin suspensions of normal skin and streptococcal skin used as control

antigens were not flocculated by serums that caused marked flocculation of the suspensions of virus-containing skin. Calf lymph and crusts from cutaneous lesions of a person with smallpox were flocculated readily by antisera against the rabbit viruses. Suspensions of brain and testicle containing vaccinia virus, although they produced potent flocculating serums, were found to be unsuitable antigens for demonstrating the specific flocculation. There is a well marked correlation between the flocculating and neutralizing titers of the various antisera, although occasionally the neutralizing titer may be quite high in the absence of any flocculating power. The flocculating antibodies are specifically absorbed by the suspensions of virus-containing skin, and it is indicated, but by no means proved, that they are also absorbed by the nonflocculatable virus brain and virus testicle antigens.

## AUTHORS' SUMMARY.

VACCINE PRODUCTION IN MEXICAN TYPHUS FEVER. H. ZINSSER and M. R. CASTANEDA, J. Immunol. **21**:403, 1931.

Rats are given subcutaneous injections of a mixture of equal parts of benzene and olive oil. Three days later the animals are given intraperitoneal injections of highly infectious guinea-pig tunica vaginalis. Three to five days after injection the animals are killed by bleeding. Through an opening in the peritoneum, scrapings of the parietal layer are made and examined for *Rickettsia*. The peritoneal cavity is washed out with a 0.2 per cent solution of formaldehyde in salt solution. Also the tunica vaginalis is removed and the *Rickettsia* content suspended in the formaldehyde solution. Twenty cubic centimeters of vaccine is obtained from each rat that is dead in from forty-eight to seventy-two hours. Though somewhat toxic, 5 cc. may be given to a small monkey with only a temporary rise in temperature.

EDNA DELVES.

STUDIES ON THE SEROLOGICAL REACTIONS OF THE FLAGELLA OF *B. TYPHOSUS*. J. CRAIGIE, J. Immunol. **21**:417, 1931.

A complement-fixation technic applicable to suspensions of the flagella of *B. typhosus* has shown that such suspensions deviate complement specifically in the presence of (a) somatic agglutinating serums, (b) pure flagellar agglutinating serums. The "antigen" which reacts with somatic agglutinating serums is heat-stable, while that which reacts with pure flagellar agglutinating serums is not completely inactivated by exposure to 100 C. for one hour. If the labilotropic agglutinin of *Felix* is identical with flagellar agglutinin, the double receptor hypothesis is of doubtful validity. The possibility that the complement-fixation technic adopted by this author is unsuited for the demonstration of fixation in which the labilotropic antibody is involved is discussed. It is probable that flagella are much more sensitive to the action of agglutinating serums than are somata and, if so, the antibody content of flagellar and somatic serums cannot legitimately be compared on the basis of agglutinin titers. If the H titer of a serum gives an exaggerated impression of the amount of H antibody present, the main arguments in favor of the double receptor hypothesis require reconsideration.

## AUTHOR'S SUMMARY.

ANTIGENIC ACTIVITY OF VARIOUS STRAINS OF *TREPONEMA PALLIDUM*. CHRISTINE E. RICE, J. Immunol. **22**:67, 1932.

Distilled water, salt solution and methyl alcohol are satisfactory solvents of the antigenic substances from all strains of *Spirochaeta pallida* studied. Extracts of certain strains with ethyl alcohol were of low antigenic activity. Although more marked fixation occurred in the presence of homologous antiserum, there was considerable cross-fixation between strains, which would suggest that, although the strains vary, they do not differ essentially in antigenic activity. Alcoholic extracts of Kroo and Klopstock strains react in the presence of syphilitic serum more

strongly than similar extracts of the Zinsser and Noguchi strains but always to a much less degree than do routine nonspecific organ extracts. Aqueous or saline extracts show little activity in such systems. From the fact that the relationship between the amounts of complement fixed by the alcoholic extracts of spirochetes and by the routine antigens remained fairly constant, it is assumed that the reaction in both cases is dependent on the lipoidal character of the preparations.

AUTHOR'S SUMMARY.

THE FORSSMAN ANTIGENS IN *B. PARATYPHOSUS* B AND *B. DYSENTERIAE* SHIGA. K. LANDSTEINER and PHILIP LEVINE, *J. Immunol.* **22**:75, 1932.

The recent studies on the properties of Forssman antigens in bacilli and their relation to the heat-stable agglutinin are suggestive of a carbohydrate nature, and a similar view has been pointed out also for the specific groupings of the Forssman haptin in animal tissues. This offers an explanation for the group reactions of a number of different substances, as it has been shown that various specific carbohydrates may contain the same sugars and sugar acids as chief constituents. In order to obtain conclusive evidence, investigations on purified material seem necessary.

AUTHORS' SUMMARY.

THE ETIOLOGY OF SERUM DISEASE. H. RUSSELL MYERS, *J. Immunol.* **22**:83, 1932.

The results reported indicate that serum disease following the injection of horse serum is not due to horse fibrinogen.

SKIN REACTIONS TO THE COLON BACILLUS AND ITS TOXIC PRODUCTS. BERNHARD STEINBERG and CATHERINE ORTH WILTSIE, *J. Immunol.* **22**:109, 1932.

Normal persons give a positive reaction to an intradermal injection of the toxic filtrate of colon bacilli and to the washed organisms. Children show a more marked reaction than adults. Patients with a long-standing colon bacillus infection react slightly, and a small percentage of the patients do not react. Negative reactions to the vaccine are more numerous than those to the soluble toxic substance. Immunization with the soluble toxic substance reduces but does not obliterate the reaction. Absence of agglutinins for colon bacilli cannot be used as a criterion for absence of other neutralizing antibodies, since titration of the agglutinins showed them to be either nil or slight in persons with negative reactions. Because normal persons give positive reactions and patients with long standing colon bacillus infection show negative or reduced reactions, the phenomenon is assumed by us to be one in which antibodies neutralize the toxic substances, both that which is soluble and that which is derived from the bacterial bodies. Our work thus far does not justify either an acceptance or a refutation of the suggestion made by some workers that the phenomenon may be allergic. It is further concluded that, under the conditions of these experiments, the cutaneous reaction for the determination of the presence of colon bacillus infection is of uncertain value.

AUTHORS' SUMMARY.

DIPHTHERIA ANTIBODIES TRANSMITTED FROM MOTHER TO CHILD. JAMES M. NEILL and others, *J. Immunol.* **22**:117, 1932.

Measurements were made of the diphtheria antibodies in the blood of a mother and baby during eighteen months following the baby's birth. Tests were made for antibacterial antibodies, as well as for antitoxin. The antibacterial antibodies were found in the serum of the baby over the period in which placentally transmitted antibodies could be expected to be retained, but owing to their passive origin they were absent in later bleedings. Control tests showed that the antibacterial

antibodies were absent in the serums of babies whose mothers did not possess them. The data on the antitoxin tests owe their interest to the high degree of immunity of the mother and to the length of time over which the blood of both baby and mother was studied.

AUTHOR'S SUMMARY.

METHOD FOR INCREASING AND PROLONGING THE PRODUCTION OF ANTITOXINS IN HORSES. G. RAMON and E. LEMÉTAYER. J. Immunol. **22**:125, 1932.

The increase in the production of antitoxin from adding to the antigens tapioca or calcium chloride is ascribed to the enclosure of the antigen in an inflammatory focus from which the antigen is absorbed gradually.

ANAPHYLAXIS TO DIPHTHERIA TOXIN. PASSIVE TRANSFER EXPERIMENTS. JAMES M. NEILL, John Y. SUGG and LURLINE V. RICHARDSON, J. Immunol. **22**:131, 1932.

Intravenous injection of diphtheric material containing sufficient toxin causes anaphylaxis in guinea-pigs previously sensitized with homologous antitoxic serum. The characteristic type of the symptoms and the passive transfer serve to identify the reaction as one of typical anaphylaxis, not to be confused with the so-called "Toxin-Ueberempfindlichkeit" of Behring. Since rigorous controls of nontoxic antigens and of antibodies for diphtheria other than antitoxin were included in the experiments, it was concluded that the toxin and antitoxin were the antigen and antibody responsible for the anaphylaxis. This conclusion drawn from the experimental data is also justifiable on a theoretical basis; for, after all, as pointed out in the discussion, there is no *a priori* reason not to expect toxin to function as other antigens in respect to production of anaphylaxis. Consequently, in this respect, at least, there is no reason to separate "antitoxinogens" from "sensitizing" antigens, nor antitoxins from "sensitizing antibodies."

AUTHORS' SUMMARY.

PRECIPITATION TEST FOR SYPHILIS. E. WEISS, J. Infect. Dis. **49**:436, 1931.

In the precipitation test, the saline solution can be successfully replaced by any one of a number of salts and acids. Salts formed by sodium and potassium with strong acids appear very satisfactory. Among the acids, sulphosalicylic and carbolic acids are most suitable. The property of precipitating proteins is not essential to flocculation of syphilitic serum. Hydrogen ion concentration, constitution, valence and molecular weight have apparently no direct bearing on the results.

AUTHOR'S SUMMARY.

EXPERIMENTAL IMMUNIZATION OF GUINEA-PIGS WITH BCG. H. J. TIEDEMANN and E. A. SCHNIEDER, Beitr. z. klin. d. Tuberk. **78**:1, 1931.

Guinea-pigs treated with BCG show on reinfection more rapid development of local nodules and delayed infection or an absence of infection of the regional lymph nodes. A definite immunity can be obtained with BCG only against such minimal doses on reinfection that not all controls develop tuberculosis. If a higher dosage of virulent bacilli is used for the test, the course of the disease is only somewhat slower in the treated animals than in the controls.

MAX PINNER.

TUBERCULIN SENSITIVITY AND ULTRAVIRUS. D. SZÜLE, Beitr. z. Klin. d. Tuberk. **78**:27, 1931.

The fact that guinea-pigs given injections of filtrates of tubercle bacilli react at times to tuberculin in skin tests does not prove anything in regard to a filtrable virus, since tuberculin, noninoculated mediums, extracts from organs of normal guinea-pigs and a suspension of colon bacilli or staphylococci produce the same type of reactions.

MAX PINNER.

## Tumors

THE MICROSCOPIC DIAGNOSIS OF CHORIOCARCINOMA, PARTICULARLY FROM CURETTINGS. K. ULEZKO-STROGANOWA, Arch. f. Gynäk. **146**:483, 1931.

The epithelium covering the chorionic villi behaves under physiologic conditions like that of a malignant new growth, multiplying rapidly, invading the uterus, opening the vessels there to obtain nutrition for the villi and to insure development of the fetus. The Langhans cells are the ones that are usually found in varying numbers in the myometrium in the early months of pregnancy. Curettings containing fragments of myometrium in which numerous Langhans cells are present, often in syncytial masses, with areas of necrosis and numerous mitotic figures in the invading cells, with the anamnesis and clinical course, clinch the diagnosis of chorio-epithelioma. Mitoses are practically never present in the invading Langhans and syncytial cells under physiologic conditions.

The occurrence of metastatic nodules in the vagina is not an indication of malignancy per se, since benign metastasis may occur. Such nodules have been examined by the author and found to consist of normal chorionic villi.

In case the microscopic appearance of the curettings is suggestive of chorio-epithelioma, and bleeding from the uterus continues, a second curetting is indicated. The author believes this does not increase the malignancy of the tumor. He doubts whether spontaneous cure of choriocarcinoma ever takes place, believing that such cases recorded in the literature are the result of erroneous diagnosis.

LAWRENCE PARSONS.

MORPHOGENETIC INFLUENCE OF A RECURRENT CARCINOMA OF THE OVARY ON THE UTERINE MUCOSA. W. ARNOLD, J. KOERNER and E. MATHIAS, Virchows Arch. f. Path. Anat. **277**:48, 1930.

The authors review the literature of the morphogenetic influence of tumors and present a report of a woman, aged 63 years, who died of a late recurrence of a carcinoma of the ovary removed twenty years previously. At necropsy there were found, in addition to the presence of carcinomatous tissue in the pelvis, typical decidual transformation of the endometrium, hyperplasia of the endometrial glands and hypertrophy of the myometrium. Since the original tumor had been removed twenty years previously, when the woman was still menstruating, the authors believe that the carcinomatous tissue left behind retained the functional properties of ovarian tissue and caused the characteristic uterine changes. In uterine bleeding after the menopause, when there is no lesion in the uterus or vagina, the presence of an ovarian neoplasm with rejuvenescent action on the uterus should be suspected.

W. SAPHIR.

THE COLOR OF THE SKIN AND EXPERIMENTAL TAR CANCER. T. SUZUKI, Virchows Arch. f. path. Anat. **277**:139, 1930.

The proportion of cancers that followed the application of tar to the skin was greater in white mice and rabbits than in black animals of the same species. In animals with skin of mixed colors, i. e., with black and white spots, tar cancers developed more frequently in the white spots than in the black ones. The author concludes that the color of the skin is an important predisposing factor in experimental tar cancer.

W. SAPHIR.

CAVERNOUS HEMANGIOMA OF THE BRAIN. W. SEHMISCH, Virchows Arch. f. path. Anat. **277**:431, 1930.

Two cases of cavernous hemangioma of the brain are reported, one presenting the typical symptoms of epilepsy, the other no clinical manifestations of organic disease of the brain. The tumors were composed of wide, cavernous blood spaces, the walls of which were partly hyalinized, calcified and pigmented by iron.

W. SAPHIR.

GANGLIONEUROMA OF THE BRAIN. J. WÄTJEN, *Vichows Arch. f. path. Anat.* **277:441**, 1930.

In a woman, aged 34, with the clinical diagnosis of tumor of the brain, necropsy revealed a tumor of the right parietal lobe. Grossly, this appeared to be a glioma; on histologic examination, it was found to contain several kinds of nervous system tissue glia, ganglion cells and nonmyelinated nerve fibrils. Giant ganglion cells with multiple nuclei were present in considerable numbers. From its structural composition the author names the tumor ganglioglioblastoma amyelinicum gigantocellulare.

W. SAPHIR.

PRIMARY MELANOMA OF THE LIVER. R. KOCH, *Virchows Arch. f. path. Anat.* **277:489**, 1930.

In the case of a woman, aged 35, a clinical diagnosis of melanoma of the liver was based on a rapidly growing tumor of the liver and a positive test for melanogen in the urine. Necropsy revealed a pigmented tumor of the liver with large amounts of melanotic pigment and in some places a sarcomatous structure, in others an alveolar, carcinomatous arrangement. There were no metastases, and no site of origin outside the liver could be found.

W. SAPHIR.

NERVE FIBRILS IN SQUAMOUS CELL CARCINOMA. W. MARTYNOW, *Virchows Arch. f. path. Anat.* **278:498**, 1930.

This is a study of the distribution of nerve fibrils in fourteen epitheliomas of the upper lip, two of the penis and one each of the tongue and upper lip. The methylene blue (methylthionine, U. S. P.) and the Golgi silver impregnation methods were used for the demonstration of the fibrils. Details of the methods used and a brief review of the findings of previous workers are given. Intercellular nerve fibrils are found in the epithelial cell nests of squamous cell carcinoma and bear the same relations to the cells as in normal squamous epithelium. They are new-formed, naked axis cylinders. In the supporting stroma of the tumor, the nerve fibrils often form dense, tangled networks. Fibrils were observed in keratinized cancer pearls; here they revealed various degenerative changes. Nerve fibrils were increased in number in the epithelium at the margin of the neoplasm. Farther away from the tumor they were normal.

O. T. SCHULTZ.

OSTEOSARCOMA IN THE RABBIT FOLLOWING RADIUM IRRADIATION. O. SCHÜRCH and E. UEHLINGER, *Ztschr. f. Krebsforsch.* **33:476**, 1931.

A platinum needle containing the equivalent of 1 mg. of elemental radium was buried beneath the periosteum of the jaw of a rabbit for twenty days. A year and a half later there developed at the site of this procedure a malignant tumor with the morphologic features of an osteogenic sarcoma.

H. E. EGGERS.

CANCER IN THE CHINESE. J. HEINE, *Ztschr. f. Krebsforsch.* **33:529**, 1931.

This report is based on somewhat limited material: a total of 525 tumor specimens received for diagnosis and 12 tumors found at 206 autopsies. Consequently, the writer can draw no far-reaching conclusions. But tumors appear to be fully as frequent among the Chinese as in western lands, and they appear to show an agreement with those of the Occident as concerns distribution, age incidence, etc.. Primary carcinoma of the liver and of the penis appears to be unduly frequent in China.

H. E. EGGERS.



ON MULTIPLE PRIMARY TUMORS. R. W. GORJAINOWA and L. M. SCHABAD, Ztschr. f. Krebsforsch. **33**:594, 1931.

Among 1,174 cases of cancer there were observed twenty-one with multiple primary malignant tumors and eighty-four in which benign neoplasms were concurrently present. An outstanding feature was that with certain primary cancers—those of the oral cavity in general and of the larynx—associated tumors were not observed. They were very rare with skin cancer, somewhat commoner with uterine and esophageal cancer and most frequent with what in general might be termed internal cancers—those of the liver, lung, stomach, colon and ovary. In general, there was observed a marked parallelism between liability to multiple tumor development and that of metastasis of the principal tumor. The writers interpret this as evidence of what they term “general cancerization.”

H. E. EGGERS.

ON THE CANCER MORTALITY OF HANOVER. P. SCHMITT, Ztschr. f. Krebsforsch. **33**:663, 1931.

This report would appear to bear out the contention that the striking increase in the crude death rates from cancer, observed almost everywhere that reliable mortality statistics are kept, is largely, if not wholly, a matter of shift of age distribution. In Hanover, the crude rate from cancer has increased in the years 1900 to 1925 from .84 per 100,000 to 12.64. But if these rates are corrected for age distribution of population, in the case of the male sex there has been an actual decrease in the years 1910 to 1925, although with females even this rate shows increase. The total rate, however, shows a reduction during that period. In Hamburg a similar reduction has occurred, while in Cologne the corrected rates for females show the reduction. The writer anticipates a further increase in the crude death rates from cancer, as the shift in age distribution of population is still in progress.

H. E. EGGERS.

CARCINOMA IN THE YOUNG. E. WILDBOLZ, Ztschr. f. Krebsforsch. **33**:681, 1931.

This report is based on 94 cases of carcinoma which occurred in persons under the age of 30 years, observed in a study of 5,536 cases of cancer. Four of these occurred during childhood. The incidence of premature cancer appears to have lessened somewhat during recent years, as from 1906 to 1915 these cases constituted 1.86 per cent of all cases of carcinoma, while from 1920 to 1929 they formed 1.79 per cent. Most frequently they were met with in the female genital organs, with the gastro-intestinal tract coming second as a site. There were no differences evident, as concerns type of tumor, progress or presence of recognizable etiologic factors, between these and cancers occurring in later years.

H. E. EGGERS.

### Medicolegal Pathology

ABORTION AND AUTOMOBILE ACCIDENTS. DUVOIR, Ann. de méd. lég. **11**:611, 1931.

The decision regarding the responsibility of trauma, both physical and psychic, must be based on the lapse of time between the accident and the abortion, the type of violence and its point of application. Damages are estimated on the basis of temporary incapacity for the duration of inactivity, and include a *pretium doloris* to compensate for the inconveniences of a fruitless pregnancy and for the disappointment over the loss of the child, according to the merits of the case. Partial responsibility was admitted in an instance of abortion, following emotional disturbances which were called forth by the destruction of the pregnant woman's property by an automobile. Permanent disability has been allowed if a puerperal infection has been followed by salpingitis or phlebitis.

E. M. BARTON.

A FOREIGN BODY IN THE HEART. DUVOIR and BELOT, *Ann. de méd. lég.* **11**:622, 1931.

A thin chip of shrapnel, 1 by 1.5 cm., which had lodged in the right ventricle of the heart, caused no serious symptoms during fifteen years, and was demonstrable only by the roentgen rays. Apparently it was firmly fixed in the ventricle. Surgical removal of such foreign bodies should be considered when embolism or progressive cardiac insufficiency is feared. When the left ventricle is the site of the loose body, it is more susceptible to surgical intervention, while the danger of embolism is greater.

E. M. BARTON.

THE INFLUENCE OF H-ION CONCENTRATION ON THE SERUM PRECIPITIN TEST. S. PICHON and C. SANNIÉ, *Ann. de méd. lég.* **11**:733 and 737, 1931.

The precipitin reaction as made by the contact or ring method is most sensitive in a  $pH$  range from 7.2 to 7.8. Extracts of blood spots on the majority of articles tested for human blood usually have a proper hydrogen ion concentration, except those of blood spots on leather, plaster, chalk and soap. Such extracts may be so acid that false negative results are produced. In such cases, the addition of buffers to bring the  $pH$  value of the extract to the range just mentioned may result in a satisfactory positive reaction.

E. M. BARTON.

ESTIMATION OF EMOTIONAL RESPONSE BY MEANS OF THE  $pH$  VALUE OF THE URINE. P. LAIGNEE-LAVASTINE and G. D'HEUCQUVILLE, *Ann. de méd. lég.* **12**:34, 1932.

The degree of emotional response during a twenty minute period of close personal questioning can be detected in spite of simulation by measuring the difference in the  $pH$  value of the subject's urine before and after the interview. The normal response is an elevation of 0.5; a rise of from 1 to 2 is considered excessive; no change indicates emotional apathy; a fall in  $pH$  value is rare and its significance equivocal. The procedure is probably as precise as psychogalvanic tests, and simpler.

EMOTIONAL TRAUMA AND EXOPHTHALMIC GOITER. M. LABRIÉ, *Ann. de méd. lég.* **12**:53, 1932.

Most of the reports of the appearance of exophthalmic goiter after emotional trauma leave doubt both as to the actual relationship of the trauma to the appearance of symptoms and as to the genuineness of the hyperthyroidism. Injuries of the sympathetic nervous system, as in wounds of the mediastinum, may be the precursors of forerunners; enlargement of the thyroid gland due to vasomotor changes or to thyroiditis may follow. Transformation of such groups of symptoms into full blown exophthalmic goiter has not been observed.

E. M. BARTON.

FATAL AUTOMOBILE ACCIDENTS. T. G. KNUDSON, *Ann. de méd. lég.* **12**:71, 1932.

In an analysis of 256 autopsies on bodies dead as a result of automobile accidents in Denmark, the mechanisms by which the lesions were produced were classified as by direct violence, by falling, by crushing and compression, and by combinations of these. The primary results of trauma accounted for 212 deaths. The causes of 30 deaths were secondary to trauma (e. g., peritonitis, meningitis); in three cases in which the autopsy revealed death from preexisting disease, the author of the accident was acquitted of involuntary homicide. This fact alone justifies a legal autopsy after every fatal automobile accident.

E. M. BARTON.

SUDDEN DEATH FROM INSUFFICIENCY OF ENDOCRINE ORGANS. J. ALBRICHT, Beitr. z. gerichtl. Med. 11:26, 1931.

Two cases of sudden death are described, which were referred to the coroner because of complete lack of a clinical explanation. In the first case, a 21 year old soldier died suddenly while dancing and singing. The autopsy revealed a persistent thymus, which weighed 36 Gm., and which measured 2 by 7 by 8.5 cm. There was no evidence of compression of any of the surrounding organs by the thymus. Microscopically, it showed diffuse hyperplasia, with many large Hassall's corpuscles. The tonsils were large, and there was marked hyperplasia of the lymph follicles of the base of the tongue, esophagus and intestinal tract. Peyer's patches were markedly enlarged, prominent and irregular. The spleen measured 3 by 11 by 16 cm., was dark red and showed prominent follicles. The aorta and the peripheral arteries were narrowed and thin-walled. Above the aortic valve, the aorta measured 4 cm. in circumference. The heart was normal in size. The suprarenal glands also were normal in size, but revealed narrow medullae. The author classifies this case as one of sudden death in an adult with a thymolymphatic constitution, a narrow aorta and a hypoplastic suprarenal system. While in many instances such a condition has been taken as being the cause of death, the author believes that only after ruling out every other cause such an explanation might be justifiable. Even though the secretion products of the thymus are not known, the author believes that the assumption of hyperthymism or dysthymism of the organism would form a simple explanation of the death in this case.

In the second case, a 23 year old soldier suffered a minor accident. Though he complained of pain in the abdomen and extremities, clinical examination revealed no satisfactory explanation for his discomfort, and the diagnosis was hysteria. A week after the accident, on his way from the window to his bed, he suddenly succumbed. The autopsy revealed no evidence of trauma. There was moderate hyperplasia of the lymphoid apparatus. The main changes were found in the suprarenal glands. They were in normal position, embedded in fat tissue, from which they were separated with difficulty. They were cylindric in shape, granular and very firm. The right weighed 3 Gm.; the left, 2.7 Gm. On section, areas of calcification and caseous necrosis were noted. No normal medullary or cortical tissue could be made out. On histologic examination, old tubercles with caseation, fibrosis and calcification were seen, but no normal suprarenal tissue. The author stated that it is interesting that in this case no evidence of tuberculosis was revealed in any of the other organs. As to the question why there were no previous symptoms related to the disease of the suprarenal glands, the author believes that there might have been accessory suprarenal tissue outside the suprarenal glands, which could have maintained the function under normal conditions, but which became insufficient as a result of the accident.

O. SAPHIR.

# Society Transactions

## PATHOLOGICAL SOCIETY OF PHILADELPHIA

*Regular Meeting, Jan. 14, 1932*

*V. H. MOON, President, in the Chair*

### AWARD OF THE WILLIAM WOOD GERHARD GOLD MEDAL.

The William Wood Gerhard Gold Medal of the Philadelphia Pathological Society was awarded to Dr. A. N. Richards, who then addressed the Society.

### RECENT DEVELOPMENTS IN THE EXPERIMENTAL STUDY OF THE FUNCTION OF THE KIDNEY. ALFRED N. RICHARDS.

Information concerning the composition of glomerular fluid and the volume in which it is formed is urgently needed both for interpreting glomerular function and as the basis for studying tubule function. Thanks to recent developments in technic of microdissection and manipulation (Barber, Chambers), it is now possible to collect fluid from individual renal units of lower animals (frogs, *Necturus*, snakes) in quantities sufficient for micro-analysis. The revolution in chemical methods for analysis of small amounts of blood and urine (Folin, Van Slyke and others) has made possible adaptations suited to the exceedingly minute amounts of fluid from single glomeruli.

Glomerular fluid from the animals named has been submitted to quantitative analysis for chloride, total molecular concentration, total electrolyte content, urea, uric acid, dextrose and injected dyes (phenol red, indigo carmine, neutral red). The results supply a basis for the conviction that the glomerular fluid is identical in composition with a protein-free ultrafiltrate from plasma.

The blood pressure in the glomerular capillaries of frogs and of *Necturus* has been measured and found to be high enough to provide ample, effective filtration pressure for the expression of a protein-free filtrate. Hence it is thought that the present state of knowledge requires belief in the filtration doctrine of glomerular function.

The criticism that the collection of glomerular fluid is equivalent to "cupping" the glomerular capillaries can be disregarded. During the past four years, the great majority of samples of glomerular fluid have been collected with the mercury leveling bulb in a higher position than the surface of the kidney by from 3 to 10 mm. The point of the pipet projects into clear, fluid-filled intracapsular space and usually does not touch the capillary tuft.

Evidence brought forward by H. L. White, by Tamura and his colleagues, and by Höber, that the glomerular process is one of secretion or that a secretory process is superimposed on filtration, can scarcely be regarded as compelling.

The volume of fluid collected from a single glomerulus in the frog has been as high as 3.5 c.mm. per hour. This figure multiplied by the total number of glomeruli gives volumes of fluid far higher than the highest collections of urine from the ureter.

Knowledge of the comparative composition and volume of glomerular fluid and ureteral urine establishes the truth of the conception of reabsorption from the tubule. Chloride and dextrose are present in glomerular fluid in as great concentration as in plasma, but are absent or nearly so from ureteral urine. Reabsorption of water is demonstrable not only by the discrepancy between the volume of glomerular collection and the volume of ureteral urine, but also by direct observation of the degree of concentration that a phenol red solution undergoes when kept stationary within a single tubule. According to White, chloride and dextrose are reabsorbed from the proximal tubule; the chief site of reabsorp-

tion of water is distal to this. Reabsorption of dextrose and chlorides and concentration of a phenol red solution occur even when the fluid in the tubules and that in the blood vessels have the same initial composition. Simple diffusion could scarcely explain this.

When the tubule is damaged either acutely by application of dilute hydrocyanic acid or mercuric chloride, or by chronic poisoning of the frog with mercuric chloride, the power of reabsorption of chloride and dextrose, the power of extruding water and the capacity of retaining phenol red are abolished. We have suggested that the most important reason for complete or partial anuria in bichloride poisoning lies in a replacement of the normal processes of active reabsorption from a selectively impermeable tubule by processes of passive reabsorption, due to osmotic pressure of proteins in the blood and lymph, through the wall of a tubule with increased but undifferentiated permeability. This explanation is similar to that made by Dunn to explain the phenomena of oxalate nephritis in rabbits.

Quantitative knowledge of the composition of the glomerular filtrate permits the calculation of the volume of total glomerular filtrate in an experimental period required to clear the blood of any constituent, the ureteral excretion of which has been measured during the period. Such calculations of the volume of glomerular filtrate necessary to clear the blood of phenol red give figures for the rate of formation of glomerular fluid which fall well within the range of rates for the collection of glomerular fluid that have actually been made. Similar calculations of glomerular clearance of neutral red necessary to account for amounts actually excreted give figures far in excess of glomerular collections that we have made. In the case of neutral red it seems necessary to postulate processes of tubular secretion. These experiments confirm the evidence of Oliver and Shevsky and indicate that in the frog secretion is a factor in the elimination by the frog of neutral red but not in that of phenol red.

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*Regular Meeting, Feb. 11, 1932*

*V. H. MOON, President, in the Chair*

**A CASE OF TOXIC ADENOMATOUS THYROID WITH A PERSISTENT THYMUS PRESENTING UNUSUAL ANATOMICAL FEATURES. R. P. CUSTER.**

The case of a 51 year old woman with the clinical features of exophthalmic thyrotoxicosis is presented. Iodine therapy and bilateral superior polar ligation failed to produce symptomatic improvement. The patient died in so-called thyroid crisis, a terminal obstruction to respiration being prominent.

Postmortem examination showed a markedly enlarged, nodular thyroid gland, the right lobe being the larger. From the superior surface of the isthmus a persistent thyroglossal duct remnant was traced to the hyoid bone. Lying beneath the manubrium of the sternum was a bilobated persistent thymus, still maintaining its fetal attachment to the thyroid through the right suspensory ligament. The thymus saddled the aortic arch between the origin of the innominate and the left carotid arteries. This mass had been interpreted clinically to be a substernal thyroid. A thyroidea ima artery the size of a crow quill arose from the ascending aorta and ascended into the neck, giving branches to the thymus and distributing its terminal radicals widely over the anterior surface of the thyroid. The aorta was hypoplastic, barely admitting the tip of the fifth finger into its lumen.

Histologic examination of the thyroid showed a toxic adenomatous structure with signs of early regression. The thymic structure was chiefly lymphocytic, corpuscles being scant. The spleen showed follicular hyperplasia; no other lymphoid structures appeared particularly active.

Persistent thymus can be differentiated roentgenographically from substernal thyroid by its triangular shadow capping the cardiac shadow. When it is present as a complication of toxic adenoma of the thyroid, radiation therapy is strongly indicated, at least as a preoperative measure.

## FOREIGN BODY (COMMON PIN) IN THE OMENTUM. GEORGE W. CUTERBRIDGE.

Mrs. M., aged 21, was admitted to the Abington Hospital on Oct. 27, 1931. For the preceding two years she had had occasional pain in the lower right quadrant of the abdomen, but not severe enough to cause her to consult a physician. She stated that it was more of a sore feeling than a real pain. The night before her admission, she had an attack of pain more severe than any of the previous ones; she called a physician, and was referred to the hospital by him with a diagnosis of acute exacerbation of chronic appendicitis.

Examination showed the abdomen to be flat, with no rigidity anywhere and only slight tenderness to deep palpation in the lower right quadrant, none elsewhere. As the diagnosis was rather indefinite, she was referred to the roentgen department for the exclusion of a possible ureteral calculus. None was found, but the film showed the presence of a common pin in the abdomen, just to the right of the fourth lumbar vertebra, which the roentgenologist believed probably to be in the intestine. Subsequent films taken on succeeding days showed considerable mobility of the pin to various positions in the lower right quadrant of the abdomen, which at first suggested that it was passing from the ileum into the cecum and would in all probability continue on through the large intestine. With this in view, the patient was discharged three days after admission and was allowed to remain at home for a week. The pin, however, did not pass, and further roentgenograms showed it still to be in the lower right quadrant of the abdomen, in slightly varying positions on films taken on succeeding days. The supposition therefore was that it was probably embedded in the wall of the intestine and would not be spontaneously evacuated. The patient was therefore readmitted to the hospital, and was operated on on November 7, through a right rectus, lower abdominal incision. Careful palpation of the lower ileum, cecum and ascending colon failed to reveal any foreign body. This was found, however, embedded in the great omentum, slightly to the right of the midline. The omentum was fairly narrow; the pin was within 1 inch (2.5 cm.) of the free border and about  $1\frac{1}{2}$  inches (3.7 cm.) from the lower edge of the transverse colon. The omentum was only slightly congested in its immediate neighborhood, was not at all rolled, thickened or adherent. No adhesions were discovered anywhere in the abdomen or was there evidence of any old perforation of the intestine. The transverse colon, however, was not eviscerated, and therefore was not thoroughly searched.

Careful questioning of the patient subsequent to operation failed to elicit from her any recollection of having at any time swallowed a pin or of having had symptoms that would suggest its working its way through the wall of the intestine. However, as she had never had any operation of any sort previously, the most plausible explanation for the condition would appear to be that she had at some time swallowed the pin, and that it had worked its way through the wall of the bowel, the small perforation thus made having closed without the production of peritonitis.

NEOPLASMS AS THE RESULT OF INHERITABLE CHROMOSOME ALTERATIONS.  
STANLEY P. REIMANN.

Slides were presented of the skins of mice and rats treated with benzyl mercaptan and other sulphydryl compounds (Hammett, F. S.: *Protoplasma* **13**:331, 1931. Reimann, S. P.: *Am. J. Cancer* **15**:2149, 1931).

The following facts and conclusions were stressed: 1. Sulphydryl increases the rate of cell division. 2. An increase in the rate of cell division increases the differentiation and organization. 3. There is a difference in the sensitivity to division of different cells. 4. Only undifferentiated epidermis cells undergo division. 5. Injury to proliferating cells leads to structurally abnormal growths.

The following conclusions relating to neoplasia were drawn:

An increased rate of cell division alone cannot lead to the formation of tumors. There must be an alteration of the differentiating and organizing chemicals in addition, and this must be transmissible from mother cells to daughter cells.

Tumors grow slower than normal tissues.

There is no such thing as dedifferentiation in respect to a cell.

The word "embryonal" or the expression "embryonal type of cell" as applied to the cells of a neoplasm is totally inapplicable and must be abandoned.

PULMONARY ASBESTOSIS, WITH THE REPORT OF TWO CASES. HAROLD L. STEWART and CARL J. BUCHER.

Asbestos, a mineral composed of silicate of magnesium, lime and iron oxide, known to classical antiquity, has become in our day an important substance in the industrial and mechanical arts. Its chemical and physical properties, its increased use and the mode of working the raw material have made it an important source of a pneumokoniosis, known as asbestosis.

Two cases of this condition are reported. The first occurred in a man who worked in an asbestos mill as a spinner for nine years. The factory was not dusty, and he wore a silver dust protector. Five years later he died of peritonitis following a cholecystectomy. At autopsy, his lungs were found to be moderately emphysematous. There was some fibrosis about the vessels, with a deposit of asbestosis bodies and pigment.

The second case is that of a man, aged 52, who worked for nine months in an asbestos factory where no precautions against inhalation of dust were taken. Seven years later pulmonary tuberculosis developed, which, in its general course, was somewhat atypical. His symptoms progressed, and he died within three years. At autopsy, he had disseminated miliary tuberculosis of the pleura and ulcerocaseous tuberculosis of the left lung with an involvement of the regional lymph-nodes. In the upper lobe of the left lung there was a huge cavity which lacked the gross characteristics of tuberculosis. By microscopic study of the lung, we were able to demonstrate ulcerocaseous tuberculosis of the left lung which reminded one of the primary complex. The lack of giant cells and other features of adult tuberculosis was apparent. The cavity in the right lung consisted of necrotic material, scant inflammatory reaction and a poorly developed fibrous tissue wall. There was marked fibrosis throughout the lung, the vessels being particularly thickened. Pigment and asbestosis bodies were scattered throughout the organ.

Attention is called to the occurrence of asbestosis bodies in the spleen and the relation between that finding and the presence of these bodies within the lumens of vessels in the lung. It is suggested that this is the mode of transportation of these particles.

These cases illustrate the lesions produced by asbestos when inhaled, the alterations in the picture of pulmonary tuberculosis when associated with asbestosis and the importance of protecting the worker from the inhalation of dust of this kind.

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CHICAGO PATHOLOGICAL SOCIETY

*Regular Monthly Meeting, March 14, 1932*

R. H. JAFFÉ, *President, in the Chair*

THE PRODUCTION OF GASTRIC AND DUODENAL ULCERS IN EXPERIMENTAL CINCHOPHEN POISONING. T. P. CHURCHILL and F. H. VAN WAGONER.

Twenty-two dogs were fed cinchophen in doses varying from 0.33 mg. per kilogram of body weight to twenty-seven times this amount. Typical acute and chronic gastric ulcers developed in seventeen of the dogs. Difficulties in administering the drug were encountered in those dogs in which no ulcers developed. In ten of the dogs, the lesion was a single gastric ulcer on the lesser curvature near the pyloric ring. The remaining seven had multiple lesions, totaling nineteen gastric and four duodenal ulcers. Ninety-three per cent of all ulcers were located along the gastric pathway.

The ulcers ranged from small superficial erosions to large chronic ulcers with indurated overhanging margins. Two of the ulcers perforated, causing peritonitis; another had penetrated the wall of the stomach, but was sealed by surrounding adhesions. Microscopically, the gastric wall showed varying degrees of involvement, from small collections of polymorphonuclear leukocytes and a hemorrhagic exudate with the mucosa still in place (the earliest stage described) to the typical large chronic ulcer dipping into the muscular coat and surrounded by fibrous tissue and inflammatory exudate.

Acute and chronic peptic ulcers, typical as to location and gross and microscopic appearance, have been produced in a large percentage of dogs by the feeding of cinchophen.

## DISCUSSION

PAUL CANNON. Was a cinchophen compound injected subcutaneously into any animals in an attempt to produce ulcers?

T. P. CHURCHILL: We have not attempted such experiments because of the insolubility of the preparations used.

## CALCIFICATION OF THE MYOCARDIUM IN A PREMATURE INFANT. M. DIAMOND.

The complete report will be published later in the ARCHIVES.

## GRANULOSA CELL HYPERPLASIA OF THE OVARY. JOHN I. BREWER and HAROLD O. JONES.

Two of the three granulosa cell growths reported were small and were limited to the medulla of the ovaries. All three growths consisted of fibrous tissue with bizarre islands and cords of epithelial cells. In serial sections, the cell masses had the shape of follicles. Some contained single large cells the size of pre-mordial ova, and surrounding others were layers of theca interna cells.

The evidence favors an origin in embryologic rests of germinal epithelium because the cell growths and the arrangement of the epithelium in fetal ovaries were similar; they occurred in the medulla and outside the ovary where there were no follicles; there was no hyperplasia of the follicular cells in the cortex, and abortive follicles and ova were present. They were follicular because the cells were similar to granulosa cells, were arranged in follicles surrounded by theca cells, and contained ova. The presence of a hormone was indicated by uterine bleeding, hyperplasia of the endometrium, formation of follicular cysts in the ovary and, as Habbe noted, development of the breasts.

The three cases reported here are probably benign. The discrete cell masses are similar to the glandular units in a benign hyperplasia of the prostate which leads to the conclusion that the masses were a hyperplasia of granulosa cells and not a true tumor growth.

The complete report will be published in the *American Journal of Obstetrics and Gynecology*.

## DISCUSSION

R. H. JARIE. Some growths of granulosa cells are tumors and produce metastases, as Dr. Brewer and Dr. Jones have stated.

## OCCLUSION OF THE GREAT VESSELS OF THE NECK IN A CASE OF SYPHILITIC AORTITIS WITH AORTIC AND INNOMINATE ANEURYSMS. LOUIS PARMACEK.

In a case of fusiform aneurysm in a severely atheromatous and syphilitic aorta, the innominate artery was also dilated to twice its normal size and entirely filled with a thrombus. Intimal proliferation narrowed the left common carotid orifice to a diameter of 2 mm., and the left subclavian to 3 mm.



## Book Reviews

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**Tumors of Bone.** By Charles F. Geschickter, M.D., Surgical Pathological Laboratory, Department of Surgery, Johns Hopkins Hospital and University, Baltimore, and Murray M. Copeland, M.D., Memorial Hospital, New York. With Forewords by Dean Lewis, M.D., Professor of Surgery, Johns Hopkins Hospital and University, and Joseph Colt Bloodgood, M.D., Clinical Professor of Surgery, Johns Hopkins Hospital and University, Baltimore. Price, \$5. Pp. 709, with 406 illustrations. New York: American Journal of Cancer, 1931.

The dedication reads: "To Joseph Colt Bloodgood whose work has formed the basis and inspiration of this book." The purpose of the book is to promote a simpler classification of tumors of the bone, increased accuracy in their diagnosis and better methods of treatment. The clinical aspects of the subject are emphasized in the forewords on the interpretation of clinical findings, by Dean Lewis, and on the rules of procedure for lesions of the bone, by the dedicatee, who also writes a chapter on therapeutic measures in lesions of the bone. The diagnostic value of the roentgen rays as well as radiotherapy and other forms of treatment for tumors of the bone receive full consideration in the discussions of the various forms of tumor of the bone. For the moment interest is centered on the grouping and description of the tumors related to osteogenesis.

This grouping is based "upon the type and phase of tissue differentiation represented by the new growths." First come the tumors derived from pre-cartilaginous connective tissue, namely: (1) osteochondroma or benign exostosis, (2) chondroma or benign chondromyxoma, (3) primary chondromyxosarcoma, (4) secondary chondromyxosarcoma and (5) osteoblastic osteogenic sarcoma.

The first and largest of these groups includes what is commonly known as exostoses, which occur most frequently at the ends of long bones between the ages of 10 and 15 years. Histogenically, they develop from normal outgrowths, derived from precartilaginous tissue and intended for tendon insertions. A distinct form with multiple outgrowths, skeletal deformities and hereditary characteristics is described as hereditary deforming chondrodysplasia.

In the chondroma or benign chondromyxoma osseous tissue is absent or sparse; the location, central rather than periosteal, is in the bones of the hands, feet, spine, ribs or sternum. These tumors, usually single and seen most frequently between the twentieth and thirtieth years, arise from supernumerary joint cartilages derived from prechondral connective tissue, strands of which become enclosed in the bone.

Groups 3 and 4, primary and secondary chondromyxosarcomas, are described as forms of periosteal osteogenic sarcoma derived from primitive perichondrium and prechondral tissue about the joints. The form called "primary" is sarcomatous from the start, and arises at tendon insertions most frequently between the fourteenth and twenty-first years, the favorite sites being the insertions of the adductor magnus in the lower femur and of the quadriceps in the upper tibia. The "secondary" form is traced to persistent prechondral tissue in a osteochondroma or a chondroma. This tumor is seen usually between the ages of 35 and 55 years in the upper humerus, the ribs or the heel. That these two forms of chondromyxosarcoma exist is not questioned, but to attempt to distinguish between them by the terms primary and secondary is bound to be confusing because of the long established use of these terms to distinguish between primary and secondary or metastatic tumors. When a myoma of the uterus becomes sarcomatous the sarcoma is not designated as secondary, and when an adenoma gives rise to carcinoma one does not speak of that carcinoma as secondary. The use of

primary and secondary in the sense used in this book with respect to chondromyxosarcoma should be stopped at once, even if it is necessary to use a circumlocution instead.

The fifth group of tumors of the bone arising in prechondral connective tissue is the sclerosing osteogenic sarcoma, which in the roentgen film is marked "by dense radiating new bone in the periosteal zone in the metaphysis of the long bones of youthful patients and is recognized as the 'sun ray' type of sarcoma of bone." Here again the favorite sites are the lower femur and the upper tibia. In adults, a similar form of sarcoma may develop in callus or in an ossifying myositis, but the suggestion of the authors to designate this form as secondary should be disregarded for the same reasons as those advanced in speaking of chondromyxosarcoma.

The other group of tumors of the bone as related to osteogenesis includes the tumors "related to subsequent cartilaginous growth," namely, chondroblastic sarcoma, osteolytic sarcoma, benign cyst of the bone and benign giant cell tumor. Chondroblastic sarcoma, a rare tumor, arises centrally in the epiphyseal regions of the lower femur, the upper tibia and the humerus during the age of puberty. From chondroblastic proliferation arise fragments of calcified cartilage, while reactive changes develop at the margins. Osteolytic sarcoma arises in the endosteum of the shaft of the long bones, mostly in young adults, and consists of large spindle cells, round osteoblasts and poorly formed osteoid tissue. Rapid destruction of bone results; hence the tendency to pathologic fracture. Benign cysts of the bone, benign giant cell tumors and related topics receive exhaustive consideration in chapters 10 to 14.

This grouping of tumors of the bone, the origin of which is related to osteogenesis, tends to promote a clearer understanding and a more precise differentiation of sarcomas of the bone. It is a step in the right direction. In the further development of this grouping, special care should be used to avoid confusion in the nomenclature in this field.

The second part of the book deals with tumors of bone that, strictly speaking, are not of osseous origin. Of these nonosseous tumors, multiple myeloma and Ewing's tumor are regarded as entities and are described fully in all important respects. The other tumors of the group are metastatic carcinoma of the bone and sarcoma in the adjacent soft parts, invading bone by direct extension. Ewing's tumor is called Ewing's sarcoma rather than endothelioma, but it is suggested for various reasons "that the tumor probably is a primary lymphoma of bone," arising within its lymphatic vessels. There are chapters on changes in the bones in lymphoma, leukemia, chloroma and Gaucher's disease, on permanent cures in sarcoma of the bone, on differential diagnosis with a roentgenographic diagnostic chart and on juvenile lesions of the bone.

There are 406 figures, all black and white, mostly halftone reproductions of photomicrographs, of photographs of gross specimens and of roentgenograms. The illustrations are of high grade. The showing of the sites of various tumors of the bone by shadings on outlines of the skeleton is commendable. The tabulation of cases of different kinds of tumors of the bone is overdone, especially in some instances, notably exostosis, the tabulation of the cases of which occupies no less than eight full pages. The laboratory numbers, in most cases not less than five figures, of cases and specimens, gross and microscopic will mean nothing at all to the ordinary reader of the book. In some way it has come about that the chapter headings in the text frequently differ from those in the table of contents. To print the references at the bottom of the pages and again in different type at the ends of chapters, if not due to mistake, is uncommendable superfluity. The style occasionally reminds one of the hurriedly prepared and superficially revised clinical lecture. There is an obvious lack of careful, systematic revision.

The book is an important contribution to the literature of tumors of the bone. It is based on a comprehensive and well organized study of these tumors by coordination of clinical, roentgenologic and anatomic methods. The results have led to a better understanding of these tumors and to improvements in their diagnosis and treatment, and at the same time they point the way to further advances.

**Asthma and Hay Fever in Theory and Practice.** Part 1. **Hypersensitive-ness, Anaphylaxis, Allergy.** By Arthur F. Coca, M.D., Professor of Immunology, Cornell University Medical College; Clinical Professor in Medicine-Elect, New York Post-Graduate Medical School; Editor of the Journal of Immunology. Part 2. **Asthma.** By Matthew Walzer, M.D., Instructor in Applied Immunology, Cornell University Medical College; Deputy Attending Physician, Clinic of Applied Immunology, New York Hospital; Chief of Allergy Clinic, Jewish Hospital of Brooklyn. Part 3. **Hay Fever.** By August A. Thommen, M.D., Lecturer in Medicine, University and Bellevue Hospital Medical College; Director of the Allergy Clinic, Medical College Dispensary, New York University. Price, \$8.50. Pp. 851, with 97 illustrations. Springfield, Ill.: Charles C. Thomas, 1931.

New books dealing with asthma and hay fever have appeared in recent years with increasing frequency. It is indeed with great pleasure that the reviewer was able to find in this most recent publication a number of qualities that not only are sufficient to justify its appearance but stamp it as a distinctive and outstanding contribution.

The subjects were treated in an extremely thorough and exhaustive manner by the three authors, each of whom was eminently fitted for his particular task. The factor of multiple contributors did not interfere with the organic structure and unity of the work, as is frequently the case with books produced by joint authors. This uniformity of opinions was probably brought about by the fact that the book serves as an outlet for a theory in this field of pathology, and its terminology is based on the theory.

While the significance of hereditary factors in this group of diseases is generally accepted and is undoubtedly of great importance, it may be questioned whether it is justifiable to base a terminology on one factor, no matter how important, particularly in view of the undeniable fact that heredity plays an important rôle in other pathologic entities. The weakness of such tightly fitting terminological definitions seems to be that they require revisions and amendments each time a new important information is gained concerning the subject.

The first part treats the theoretical phases, in 106 pages, and serves well as an admirable theoretical introduction into the fundamentals of the field. There are 376 titles included in the bibliography. An error crept into the third chapter, in which 22 reference numbers to the bibliography are wrong.

Asthma is discussed in 348 pages. A full bibliography with 1,077 titles is appended. The detailed presentation of the methods of testing for hypersensitive-ness, in 57 pages, will prove of great value to all actively engaged in the work. The last chapter on "Atopens and Other Excitants," signed by the second author jointly with Katherine Bowman, is a veritable mine of information concerning the innumerable forms in which the various offending substances are disguised. The authors will earn the gratitude of all those who have ever tried to trace the strange metamorphosis of some such materials in the course of the various industrial applications.

In analyzing the results of the various therapeutic procedures, great emphasis is laid (and justly so) on the uselessness and even harmfulness of most surgical procedures on the nose and throat in conditions of hay fever and asthma. Too much cannot be said about this.

The danger of constitutional reactions is discussed at length in this and in the third part of the book. Case reports and data concerning this insidious and dreaded occurrence are distributed in various journals, and a summary of present-day knowledge on this matter as it is here presented will prove valuable. To quote verbatim: "Fatalities from testing nowadays, in almost every instance, can be traced to an error in procedure or a mistake in judgment which a more thorough knowledge of the subject or wider experience would have prevented."

The 15 excellent rules on pages 347 and 348 ought to be posted in every room in which allergic patients are tested and treated. Adherence to them would prevent much evil.

In the discussion of the opinions of van Leeuwen concerning the importance of human dander for the causation of asthma, the author takes a negative stand, basing it on theoretical grounds that "It is contrary to all immunologic principles for any animal to become anaphylactically hypersensitive to its own products unless these have become so altered as to lose their identity and specificity." Such a stand is not correct any more, since Krusius succeeded in sensitizing guinea-pigs to their own lens protein, and others (Hertle and Pfeiffer) seem to have achieved the same with kidney protein.

The third part, of 300 pages, deals with hay fever. A well written, historical review of 29 pages introduces the subject. One is particularly impressed by the large number of important contributions made by physicians who had suffered from the disease. More than half of the part on hay fever is given to a detailed discussion of the botanical phases of the subject. There is no denying that such knowledge is essential for the physician who is to diagnose and to combat the disease scientifically; however, the reviewer cannot help feeling that some subjects could have been treated in less space without loss in essential information. To illustrate with one example: The longevity of seeds is discussed in 9 full pages. The pages make extremely interesting reading, but include a great deal that is not essential. It is hoped that the large mass of botanical information will not act as a deterrent. Many of the 95 figures representing plants, flowers and pollens are unsatisfactory, and some are quite useless if they are to be used for purposes of identification. Many of the pictures, particularly in the chapter on weeds, are too small, too crowded and lack too many details to be of use to the nonbotanist. The general index of 42 pages and the special index of atopens and excitants of 9 pages are excellent.

The book will undoubtedly prove valuable not only to those actively engaged in the treatment for asthma, hay fever and allied diseases, but also to every physician seeking information.

A shortcoming for which the authors are not to be blamed is the unsatisfactory print on some pages, mainly in the first part, making the reading of those pages somewhat difficult.

**Recent Advances in Bacteriology and the Study of the Infections.** By J. Henry Dible, M.B. (Glas.), F.R.C.P., Professor of Pathology in the University of Liverpool; Late Professor of Pathology in the University of London, and Professor of Pathology and Bacteriology in the Welsh National School of Medicine. Second edition. Price, \$3.50. Pp. 476, with 29 illustrations. Philadelphia: P. Blakiston's Son & Co., 1932.

The first edition of this book received a favorable review (ARCH. PATH. 7:763, 1929) and was commended as a helpful summary of recent progress in bacteriology. The book now appears in a revised and enlarged form. New sections have been added on brucellosis, flocculation tests for syphilis, the *Salmonella* group and the antigenic structure. The number of pages has grown from 363 to 476, and of illustrations from 22 to 29. The confident generalization in the following statement on page 438 arouses question as to its reliability: "It is common knowledge that media upon which bacteria have grown become unsuitable for the propagation of other strains of the same organism. A broth which has been utilized for the growth of *B. coli* will not, when filtered free from these organisms, serve as a medium for a fresh culture of the same bacillus. An agar slope upon which streptococci have been grown will not serve as a medium for a second streptococcus after the colonies of the first have been washed away." The recent important contributions to the study of the specificness of the scarlet fever toxin and other streptococcal toxins are not discussed.

## Books Received

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HERTZLER'S MONOGRAPHS ON SURGICAL PATHOLOGY: SURGICAL PATHOLOGY OF THE FEMALE GENERATIVE ORGANS. By Arthur E. Hertzler, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kan.; Professor of Surgery, University of Kansas. Price, \$5. Pp. 346, with 285 illustrations. Philadelphia: J. B. Lippincott Company, 1932.

IMMUNOLOGICAL STUDIES IN REPTILES AND THEIR RELATION TO ASPECTS OF IMMUNITY IN HIGHER ANIMALS. By E. Grasset, M.D., Medical Serologist, Serum Department, and A. Zoutendyk, Senior Technical Assistant, Serum Department, South African Institute for Medical Research, Johannesburg. Publications of the South African Institute for Medical Research. Volume IV, No. 29. Paper. Pp. 377 to 460, with 28 illustrations. Johannesburg: South African Institute for Medical Research, 1931.

THE VALUE OF TUBERCULIN TESTS IN MAN, WITH SPECIAL REFERENCE TO THE INTRACUTANEOUS TEST. By P. D'Arcy Hart, Medical Research Council, Special Report Series, No. 164. Price, 2 shillings, net. Pp. 136. London: His Majesty's Stationery Office, 1932.

DIE THEORETISCHEN GRUNDLAGEN UND DIE PRAKTISCHE VERWENDBARKEIT DER GERICHTLICH-MEDIZINISCHEN ALKOHOLBESTIMMUNG. Von Prof. Dr. E. M. P. Widmark, Mediz-chem. Institut der Universität Lund, Schweden. Neue Folge, Heft 11. Fortschritte der naturwissenschaftlichen Forschung. Herausgegeben von Prof. Dr. Emil Abderhalden. Paper. Price, 14 marks. Pp. 140, with 59 illustrations. Berlin: Urban & Schwarzenberg, 1932.

REPORT OF THE MEDICAL RESEARCH COUNCIL FOR THE YEAR 1930-1931. Committee of the Privy Council for Medical Research. Presented by the Lord President of the Council to Parliament by Command of His Majesty, February, 1932. Price, 2 shillings, 6 pence, net. Pp. 153. London: His Majesty's Stationery Office, 1932.

ENTSTEHUNG, ERKENNUNG UND BEHANDLUNG INNERER KRANKHEITEN. Von Dr. Ludolf Krehl, Professor in Heidelberg. Erster Band. Die Entstehung innerer Krankheiten: Pathologische Physiologie. Vierzehnte Auflage. Price, 39.60 marks; Bound, 42 marks. Pp. 716. Berlin: F. C. W. Vogel, 1932.

CYTOLOGY AND CELLULAR PATHOLOGY OF THE NERVOUS SYSTEM. Edited by Wilder Penfield, Professor of Neurology and Neurosurgery, McGill University, Montreal. Three volumes. Cloth. Price, \$30, net. Pp. 1280, 886 illustrations, 15 in color. New York: Paul B. Hoeber, Inc., 1932.

DER NEUBAU DES PATHOLOGISCHEN INSTITUTS DER UNIVERSITÄT MÜNCHEN. Geh. Medizinalrat Universitätsprofessor Dr. M. Borst und Ministerialrat Dr. T. Kollmann. Pp. 48. Munich: Kunst im Druck G.m.b.H. 1932.

THE WISDOM OF THE BODY. By Walter B. Cannon, M.D., ScD., LL.D., George Higginson Professor of Physiology, Harvard Medical School. Price, \$3.50. Pp. 312. New York: W. W. Norton & Company, Inc., 1932.

## TUMORS OF MUSCLE TYPE

REPORT OF A GROUP OF CASES, WITH SPECIAL REFERENCE TO  
METASTASIS OF LEIOMYOSARCOMA TO THE BRAIN.

J. STANLEY COHEN, M.D.

PHILADELPHIA

Muscle tumors have been termed leiomyomas, or smooth muscle tumors, and rhabdomyomas, or striated muscle tumors. They may be benign or malignant.

The incidence of muscle tumors varies with the type. Benign myoma occurs very frequently, especially in the uterus. Gusserow<sup>1</sup> stated that 38.8 per cent of all women between the ages of 30 and 40 have myoma uteri. The tumors may be submucous, subserous and intramural in location, and are usually multiple. They may occur in the cervix, vagina, round ligaments, broad ligaments and pelvic fasciae. Reports of the incidence of malignant leiomyoma vary. Frequently statistics of its occurrence are based on the discovery of local areas of increased cellularity in the tumor, without evidence of general symptoms, metastasis or recurrences. As a result of this method of tabulation, reports are made in which it is asserted that 10 per cent of all uterine myomas are malignant. Leiomyosarcoma is much rarer than is generally believed. In twenty years of observation, Ewing<sup>2</sup> encountered three malignant uterine myomas with general metastasis and two with local recurrence. Gardner<sup>3</sup> found one case of leiomyosarcoma in the Boston City Hospital in twenty years. This coincides with the finding of two cases among 18,077 necropsies in the Philadelphia General Hospital in the course of eleven years. Leiomyosarcoma occurs usually after the menopause, though Ewing stated that polypoid myoma of the cervix is usually malignant at all ages.

Rhabdomyoma occurs infrequently. It is usually found in early life. The most common sites are the kidney, heart, vagina, cervix and testicle. Rarer sites are the esophagus, stomach, tongue, parotid gland, breast, prostate and skeletal muscles of the body. Rhabdomyosarcoma is seldom encountered.

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Submitted for publication, Jan. 18, 1932.

From the Division of Pathology of the Laboratories of the Philadelphia General Hospital.

1. Gusserow: *Die Neubildungen des Uterus*, ed. 1, Stuttgart, Ferdinand Enke, 1885, p. 269.

2. Ewing: *Neoplastic Diseases*, ed. 2, Philadelphia, W. B. Saunders Company, 1922, p. 207.

3. Gardner: *J. M. Research* 36:19, 1917.

## ETIOLOGY

No definite proof regarding the etiology of muscle tumors has been presented. The cell inclusion theory of Cohnheim<sup>4</sup> is the one most commonly mentioned. He derived his theory of the origin of all tumors from observation of a case of congenital myosarcoma of the kidney while he was working at Breslau.

Embryologically, muscle tissue arises from mesoderm. Smooth muscle arises from undifferentiated mesenchyme. Figures 1 *A* and *B* shows the source of this tissue. The stellate cells of the mesenchyme, shown in figure 1 *C*, become spindle-shaped and are connected by cytoplasmic bridges. Figure 1 *D* shows the coalescence of the granules in

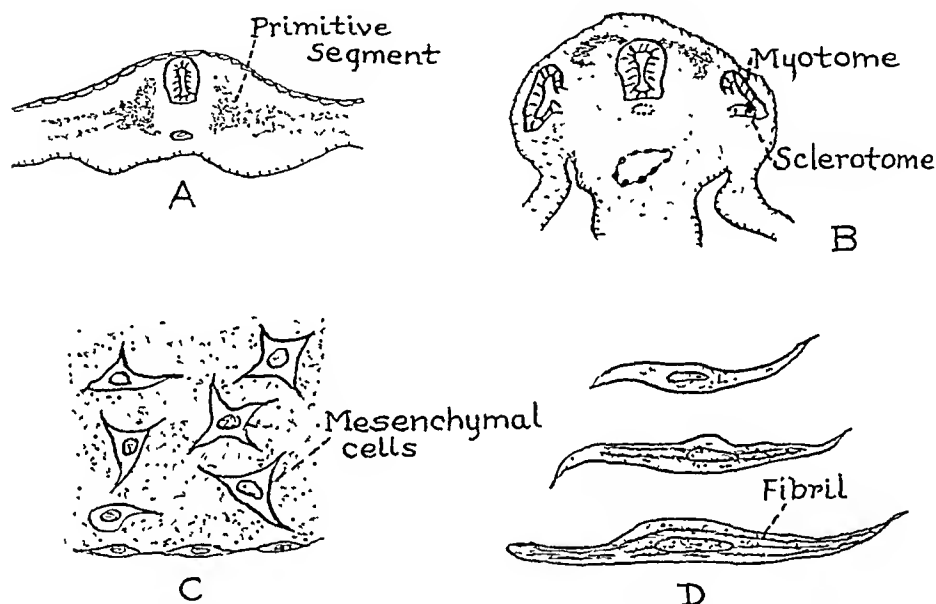


Fig. 1.—Diagrammatic representations modified from Prentiss and Arey (Embryology, ed. 2, Philadelphia, W. B. Saunders Company): *A*, chick embryo at two days; *B*, human embryo at three weeks; *C*, mesenchymal tissue, and *D*, developmental myoblast.

the superficial cytoplasm of the myoblast to form coarse, noncontractile myoglia fibrils. Later, these divide longitudinally to form fine myofibrils, thus giving the cell its longitudinally striated appearance. The cytoplasmic processes develop into white connective tissue fibers, which bind the smooth muscle cells into bundles.

Prentiss and Arey<sup>5</sup> listed the following methods of increase in the number of smooth muscle cells in the embryo: (1) formation of new cells from mesenchyme, (2) transformation of interstitial cells into

4. Cohnheim: Cohnheim's Lectures, London, New Sydenham Society, 1889, sect. 1, mem., p. XIII.

5. Prentiss and Arey: Embryology, ed. 2, Philadelphia, W. B. Saunders Company, 1918, p. 292.

muscle fibers and (3) multiplication of their nuclei by mitosis in the more advanced fetal stage. Thus, smooth muscle tumors may arise in the adult by the unrestrained growth of misplaced persistent mesenchymal cells.

Ewing<sup>6</sup> stated that the widespread occurrence of myomas in mature life and the presence in many cases of heterotopic inclusions point clearly to an embryonal origin. He believes that pure fibromyoma uteri results from the malformation of the genital organs from the müllerian ducts. Rosger<sup>7</sup> and Kleinwachter<sup>8</sup> believe that myoma arises from the walls of blood vessels. Muller<sup>9</sup> and Larkin<sup>10</sup> are of the opinion that myoma of the kidney arises from fragments of the capsular tissue.

Embryologically, striated muscle arises from the myotome, shown in figure 1 *B*. The muscles of the head are exceptions; they arise from mesenchyme. There are two theories concerning the formation of striated muscle fibers: 1. The myoblast of the myotome elongates and by repeated mitotic division becomes multinucleated. 2. The myoblasts unite to form single, elongated muscle fibers.

Some investigators believe that the micelle of the myofibril occurs in the ovum; others,<sup>11</sup> that the myofibrils arise from the mitochondrial rods and filaments, without a granular stage. Godlewski<sup>12</sup> believes that the fibrils pass through a granular stage as described in the development of the smooth muscle cell. Wolbach<sup>13</sup> thinks that the fibrils arise by connection of centrioles. Baldwin<sup>14</sup> regards the myofibril as a differentiated product of the muscle cell, the homologue of the connective tissue fiber.

Three theories of the etiology of rhabdomyoma are possible: (1) the cell inclusion theory of Cohnheim, (2) that of development of the striated muscle cell from the fibroblast through metaplasia and (3) that of differentiation from the smooth muscle cell. The latter possibility is suggested by the work of Carey,<sup>15</sup> who succeeded in transforming the normal smooth muscle cells of the urinary bladder into striated muscle by repeated filling and emptying of the bladder. On the other hand, Reyder stated that rhabdomyoma of the heart is persistent embryonal tissue and not a true neoplasm.

6. Ewing (footnote 2, p. 213).

7. Rosger: *Ztschr. f. Geburtsh. u. Gynäk.* **18**:131, 1890.

8. Kleinwachter: *Ztschr. f. Geburtsh. u. Gynäk.* **9**:68, 1883.

9. Muller: *Virchows Arch. f. path. Anat.* **145**:339, 1896.

10. Larkin: *J. M. Research* **6**:25, 1901.

11. Morceau: *Bibliographie Anatomique*, Paris, 1902, vol. 10, p. 1.

12. Godlewski: *Arch. f. mikr. Anat.* **3**:60, 1902.

13. Wolbach: *Anat. Rec.* **37**:255, 1928.

14. Baldwin: *Ztschr. f. allg. Physiol.* **14**:146, 1912.

15. Carey: *Am. J. Anat.* **29**:341, 1921.



## LEIOMYOMA

Histologically, leiomyoma consists of irregular bundles of cells, frequently arranged in whorl formation. The individual cells are spindle-shaped, but rather thicker and shorter than normal smooth muscle cells. They have centrally located, relatively long, oval, chromatic nuclei. The cytoplasm is less acidophilic than that of the normal analogue. Mallory<sup>16</sup> considered the myoglia fibrils described by Heidenhain the most important factor in the differentiation of smooth muscle cells from

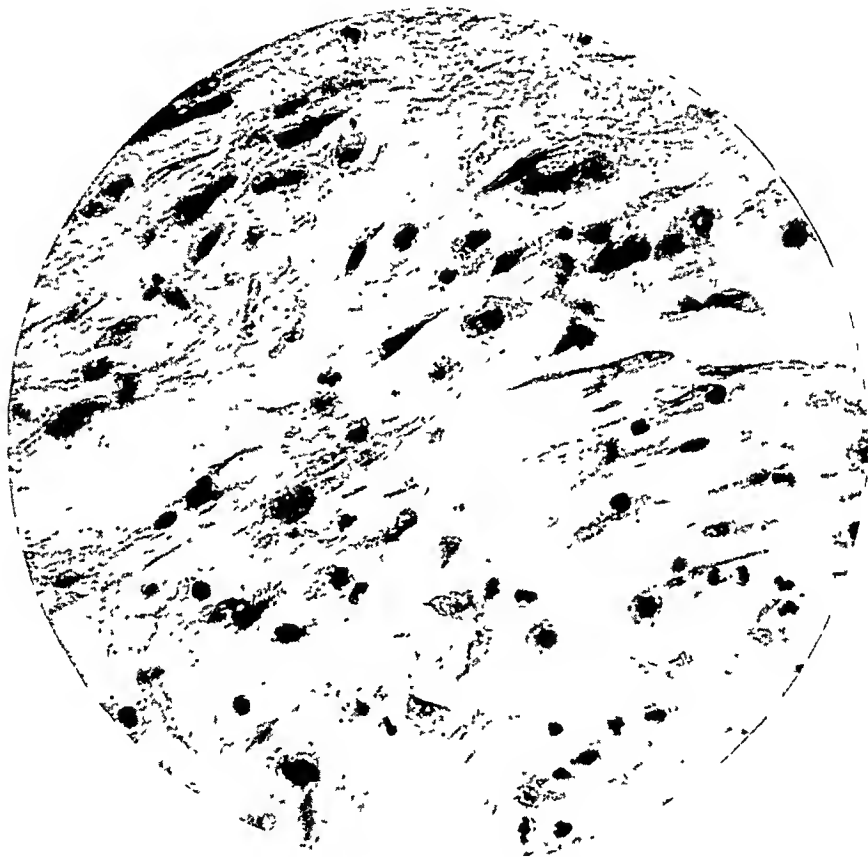


Fig. 2.—Photomicrograph showing intracellular fibrils of smooth muscle cells;  $\times 520$ .

fibroblasts. These fibrils, situated within the limiting membrane, fuse at the end of the cell to form coarse fibrils, while the fibrils of the fibroblast spread out in fan shape some distance from the cell. Figures 2 and 3 picture this essential difference. Differential stains, such as Mallory's phosphomolybdic acid, aniline blue, orange G or van Gieson's alum hematoxylin, acid fuchsin, trinitrophenol, can be used with assurance in distinguishing the muscle from the connective tissue.

16. Mallory: Principles of Pathologic Histology, ed. 1, Philadelphia, W. B. Saunders Company, 1920, p. 305.

The following five cases of leiomyoma were found incidentally at postmortem examination:

CASE 1.—A white woman, aged 70, died of intraventricular hemorrhage. In the kidney was found a tumor composed of smooth muscle cells arranged irregularly. Histologically, it was a leiomyoma. It had produced no symptoms.

CASE 2.—A white man, aged 80, died of bronchopneumonia and hemorrhagic infarction of the kidney. A small, gray nodule was found in the cardiac end of the stomach. It was 1.8 cm. in diameter and shelled out readily. Histologically, it was a leiomyoma. It had produced no symptoms.



Fig. 3.—Photomicrograph showing the fan-shaped arrangement of extracellular fibroblastic fibrils;  $\times 520$ .

CASE 3.—A white woman, aged 79, suffered from multiple neurologic phenomena, shown by necropsy as due to convolucional atrophy and arteriosclerosis of the basal ganglions. A round mass was found in the ileum projecting into the lumen. The overlying mucosa was intact. No surrounding induration was present. Histologic examination showed this to be a leiomyoma.

CASE 4.—A colored woman, aged 53, died of diabetic gangrene of the right leg. Two small nodules were found in the cardiac end of the stomach. They were well circumscribed and lay loosely under the mucosa. Histologically, they were leiomyomas.

CASE 5.—A white woman, aged 76, died of cerebral hemorrhage. A tumor 8 mm. in diameter was found movable in the muscle of the gastric wall. It was a soft, well circumscribed leiomyoma.

#### LEIOMYOSARCOMA

Arthur Hertzler<sup>17</sup> expressed the belief that malignancy in myoma of the uterus is due to hemorrhage into the tumor. He listed the changes as follows: (1) obliterating endarteritis, (2) hyaloid degeneration of the wall of the blood vessel, (3) degeneration of the surrounding tissue, (4) free hemorrhage and (5) rapidly developing sarcoma from hemorrhagic tissue. This idea has met with much objection.

The cells of the malignant leiomyoma are shorter and rounder than those of the benign tumor. The nuclei are massive and hyperchromatic. Giant cell forms may exist. The stroma is scanty. The walls of the blood vessels are defective. Evans<sup>18</sup> believes that the occurrence of many mitotic figures is a reliable sign of malignancy. Proper and Simpson<sup>19</sup> classified malignant leiomyomas into three groups: (1) those closely resembling leiomyomas, (2) those having short spindle-shaped cells with oval nuclei and (3) those showing great variation in cell morphology. He believes that malignancy increases progressively through the groups.

CASE 6.—A colored woman, aged 47, was admitted to the hospital complaining of intermenstrual bleeding. The last menstrual period occurred in October, 1930. In November, 1930, she passed large clots. Following this, the periods were irregular, and a thick discharge of foul, yellow material occurred, occasionally intermixed with blood. Cramps occurred in the abdomen after meals, inducing nausea and vomiting. In January, 1931, the patient noted dyspnea on exertion, severe night sweats and diurnal and nocturnal frequency of urination.

The lungs were normal. The heart was slightly enlarged to the left. Presystolic and systolic murmurs could be heard over the entire precordium. The abdomen was distended by a hard, nodular mass, which arose in the pelvis and extended to the umbilicus. It appeared to be a part of the uterus.

Roentgen study revealed a dense, circular shadow in the left lower hemithorax at the level of the ninth rib in the midclavicular line. The right dome of the diaphragm was higher than normal and was obliterated by the presence of fluid.

Necropsy revealed multiple white, firm nodules in the uterus, enclosing areas of soft hemorrhagic tissue. There were secondary nodules in the lungs and liver. The nodules in the lungs were firm, white and circumscribed; that in the liver was necrotic, and had ulcerated through the diaphragm into the right lung.

Histologically, the cells of the soft hemorrhagic areas in the uterus were larger than those of the surrounding tissue. The nuclei were fairly large and varied in shape, some being spindle-shaped, some round and some oval. A few bizarre cells contained multiple nuclei. The cytoplasm was small in amount and irregularly fibrillar.

17. Hertzler: J. A. M. A. **71**:1040, 1918.

18. Evans: Surg., Gynec. & Obst. **30**:225, 1920.

19. Proper and Simpson: Surg., Gynec. & Obst. **29**:39, 1919.

In reviewing the literature, I was unable to find a record of a myoma occurring as a primary or as a secondary neoplasm of the brain. If this survey is correct, the following report of a case describes the first recorded instance of leiomyosarcoma that metastasized to the brain:

CASE 7.—An emaciated, senile white man of 65 was admitted to the hospital, complaining that he had had "failing health" since the occurrence of a "stroke" in 1928. Further history could not be obtained.

The liver was enlarged to a level 4 fingerbreadths below the costal margin. A hard, fixed, circumscribed nodule about 7 cm. in diameter could be felt at the lower border of the liver, about 4 cm. to the right of the umbilicus. It moved with respiration.



Fig. 4.—Photograph showing nodule of leiomyosarcoma in the occipital lobe of the brain.

Roentgen examination revealed a slight six hour retention due to a mass in the stomach, which was interpreted as carcinoma.

The urine contained a trace of albumin and many leukocytes. The red blood cell count was 4,400,000; the hemoglobin content was 12.7 Gm.; the leukocyte count was 17,200, with 81 per cent polymorphonuclears. The Wassermann reaction of the blood was negative. The blood sugar determination was 113 mg., and that of blood urea nitrogen was 20 mg. Fractional gastric analysis showed no free hydrochloric acid; total acid was 15, 15, 19, 22, 19, 17, 22 and 29 mg. Lactic acid was present.

Necropsy revealed a primary leiomyosarcoma of the left kidney with metastases to both lungs, right kidney, suprarenal glands, ileum, mediastinal and mesenteric lymph nodes and brain. The right kidney weighed 680 Gm. It was irregular and

nodular, but freely movable in the perirenal fat. It was firm and cut with increased resistance. It was homogeneous and of a uniform yellow color. In the upper pole was a round area 4 cm. in diameter, composed of deep yellow, necrotic material, putty-like in consistency. No renal tissue was present that could be recognized as such grossly. The nodules in the lung were numerous, varying in size from 4 mm. to 3 cm. in diameter. They were all firm, resistant and yellow.

The brain weighed 1,520 Gm. It was large but of normal shape. The pia-arachnoid was thickened, and showed fibrosis of the interpeduncular space. The cortical convolutions were swollen, and the blood vessels were congested. The pons, medulla and cerebellum appeared normal. The ventricles were definitely

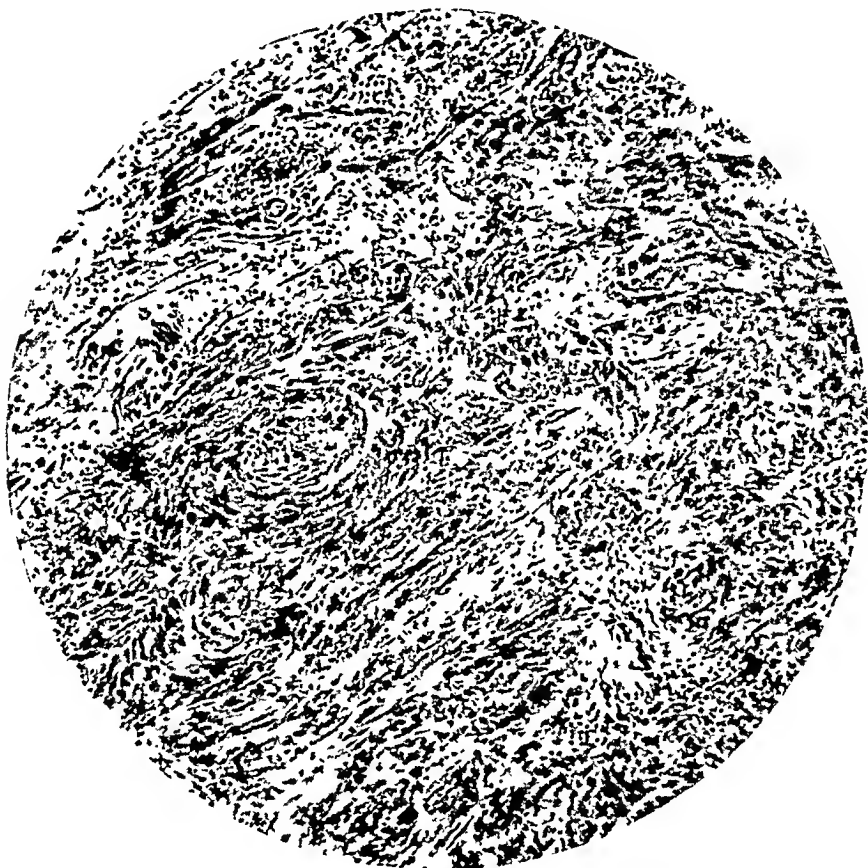


Fig. 5.—Photomicrograph of leiomyosarcoma of the brain showing whorl formation;  $\times 69$ . For high power magnification, note figure 2.

dilated. The basis pontis contained a scar on the right side resulting from an old thrombotic lesion. Practically the entire right half of the basis pontis was degenerated. When the left occipital lobe was cut through, a nodule was seen deep in the subcortex. This lay immediately posterior to the calcarine cortex, and the softening around it involved the latter structure. The nodule measured 2 cm. in longitudinal diameter and 0.5 cm. in transverse. It was firm and yellowish green, and showed central necrosis. It appeared encapsulated owing to softening of the white matter about it. This is seen in figure 4. Examination of the tumor under low power, with the 16 mm. objective, presented a whorl appearance, shown in figure 5.

Examination under high power magnification, with the 4 mm. objective, showed the cells to be of irregular spindle shape. With toluidine blue stain, the cytoplasm stained light blue and presented longitudinal striations. The nuclei were oval, elongated and centrally located, and contained a central spherical nucleolus. Both stained dark blue. Figure 2 illustrates these cells.

#### RHABDOMYOMA

Histologically, the picture of rhabdomyoma varies, depending on the degree of maturity of the cells comprising the tumor. The mature type



Fig. 6—Photomicrograph showing rhabdomyosarcoma in the left suprarenal gland;  $\times$  69.

of tumor consists of irregular bundles of striated muscle; the immature type, of single cells or small groups of cells with prominent nuclei and scanty cytoplasm. The cells are shorter and rounder, and resemble smooth muscle cells. Cross-striations may be rare, but areas can always be found where the cells show characteristic striations. The mature type of cell is generally found in the benign tumor; the immature type is more likely to be malignant. The stroma may be loose adult connective tissue or a more embryonal myxomatous or sarcomatous type. Vascularity is usually prominent. The following report represents the

only case of rhabdomyosarcoma found among 18,077 necropsies at the Philadelphia General Hospital in eleven years.

CASE 8.—In November, 1929, a white woman, aged 18, complained of a loss of sensation over the left hip. One month later, she began to have pain in the left side of the chest and noticed lumps over the entire body. During July, 1930, a gradual onset of paralysis of the legs occurred. In January, 1931, amenorrhea began.

Both pupils were dilated. Cervical adenopathy was present. There was increased vocal resonance, and râles were heard throughout the chest. The heart was displaced upward; the rate was rapid and irregular, but no murmurs could



Fig. 7.—Photomicrograph showing cross-striations in rhabdomyosarcoma;  $\times$  520.

be heard. The blood pressure was 104 systolic and 72 diastolic. Ascites was present. Four large nodes were palpable in the anterior abdominal wall. The muscles of the legs were atrophic. The deep tendon reflexes were absent. The course of the illness was progressive, and the patient died on Sept. 1, 1931.

Necropsy revealed a white woman, 170 cm. in length and 75 pounds (34 Kg.) in weight, with scoliosis to the right and bed sores over the sacrum. The heart weighed 200 Gm. and contained numerous nodules throughout the myocardium. One nodule, 1 cm. in diameter, was present in the posterior aspect of the septum, there was another in the interventricular septum near the apex. They were glistening, white, smooth and friable, resembling pearls. The lungs showed numerous abscesses. The left kidney contained a large, nodular tumor in the upper pole, 3

cm. in diameter. It was irregularly round, soft, glistening and friable. The right kidney was the seat of multiple abscesses. The capsule of the spleen contained many patches. The liver showed chronic, passive congestion. The small bowel had numerous small tumors situated in the serous coat. A large tumor involved the pancreas, diaphragm, omentum, right kidney, aorta, spinal column, dura of the cord, left suprarenal gland and left ovary.

Histologically, the tumor cells of the left suprarenal gland were most characteristic. They corresponded to the immature type of rhabdomyosarcoma, but showed cross-striations. The tumor cells of the other organs showed a rather marked degree of necrosis. Figures 6 and 7 show the metastatic tumor in the suprarenal gland under low and high power objectives.

#### SUMMARY

The incidence of muscle tumors varies with the type. Leiomyoma occurs frequently, especially in the uterus. Leiomyosarcoma is less frequent, according to my studies, than is generally claimed. What appears to be the first case of metastasis of leiomyosarcoma to the brain is described. Rhabdomyoma occurs very rarely. Rhabdomyosarcoma is rare. The diagnosis of muscle tumors depends on close observation of the type of cell, i. e., the finding of spindle-shaped cells arranged in whorl formation or of cross-striations in large, irregular cells. Mallory's and van Gieson's differential stains are of diagnostic aid. The possible theoretical origins are enumerated.



# LOCAL TISSUE IMMUNITY

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Immunologic investigations of recent years place increasing emphasis on the direct part played by the mesenchymal tissues in resistance to infectious diseases, and as a result cellular reactions of inflammation are being studied with renewed interest in the attempt to understand their fundamental significance in the general mechanism of defense. The idea of localization of infection as a definite defensive factor has assumed greater importance in the minds of many students of the problem, although the importance of general humoral elements is not denied. The conception has developed that increased resistance to certain infectious agents may be largely local, due to a more effective response of local tissues to the invading micro-organism. This notion, while not new, has been emphasized particularly by Besredka,<sup>1</sup> who has stimulated renewed interest in the problem of local immunity. New facts have resulted from this interest which not only strengthen the view that such an immunity actually exists, but also help to explain its mechanism.

In this paper experiments are reported which further support the idea that local immunity can be acquired.

There is at present some confusion regarding what is meant by the term local immunity. Besredka,<sup>2</sup> for example, defined it as "an immunity without the obligatory participation of antibodies." Gay,<sup>3</sup> on the other hand, defined the condition as a "locally superior mechanism for the disposal of a particular micro-organism," and stated that it may be demonstrated "either by the local presence of antibodies before their appearance elsewhere in the body, by their local presence in greater concentration than elsewhere, or by a superior method of direct disposal of bacteria in the particular area in question." Opie<sup>4</sup> interpreted local immunity after a previous sensitization to a specific antigen or bacterium as a local hypersensitivity that is manifested by an acute inflammation having the essential features of a local anaphylactic reaction, and tending to retard the entrance of bacteria or their noxious products

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1. Besredka, A.: *Compt. rend. Soc. de biol.* **88**:1273, 1923.

2. Besredka, A.: *Local Immunization*, Baltimore, Williams & Wilkins Company, 1927.

3. Gay, F. P., in Jordan, E. O., and Falk, I. S.: *The Newer Knowledge of Bacteriology and Immunology*, Chicago, University of Chicago Press, 1928.

4. Opie, E. L.: *J. Immunol.* **17**:329, 1929.

into the blood stream. This retardation, he assumed, is brought about by the local accumulation of antibodies, which fix the antigen or bacteria at the site of inoculation, thus localizing the infectious agent and preventing its dissemination to more vital organs.

Regardless of differences in point of view concerning the actual mechanism of local immunity, most of the workers agree as to the existence of a localized condition that brings about an increased resistance to a specific micro-organism. This does not necessarily eliminate or minimize the importance of a generalized response.

#### HISTORICAL EVIDENCE FOR THE EXISTENCE OF LOCAL IMMUNITY

Fehleisen<sup>5</sup> in 1883 was probably the first to note evidence of experimental local immunity. He observed a locally increased resistance while reproducing erysipelas in animals and human beings who had recovered from previous attacks of the disease. Meierowitsch<sup>6</sup> in 1888 found that rabbits which had recovered from one attack of experimental erysipelas were protected from a second attack for from one to two months. As evidence that the immunity acquired after erysipelas is general as well as local, Roger<sup>7</sup> produced an attack of erysipelas in one ear of a rabbit, and after recovery had taken place, produced a second attack in the opposite ear, and found that it was followed by a rapid recovery or the formation of a small abscess. On the other hand, Gromakowsky<sup>8</sup> called attention to the absence of immunity against the streptococcus of erysipelas in the peritoneal cavity of animals previously immunized in the skin.

Cobbett and Melsome<sup>9</sup> produced both local and general immunity against erysipelas by subcutaneous inoculations of a pure culture of living streptococci into the ears of rabbits. They demonstrated the presence of local immunity by comparing the effects of inoculations of living streptococci into the ears of rabbits that had recovered from previous inoculations of the same micro-organism, with those of similar inoculations into normal rabbits. The response in the previously inoculated rabbits was manifested by an early local inflammatory reaction that subsided in from one to four days and the absence of general toxic effects. The control rabbits, on the other hand, presented a slow, progressive inflammatory reaction accompanied by general malaise and in many instances by death. The existence of a generalized immunity was demonstrated in two ways, first by producing an attack of erysipelas in the right ear and a few days after recovery producing a second attack in the left ear. The reaction was compared in extent and out-

5. Fehleisen: *Aetiologie der Erysepelas*, Berlin, T. Fischer, 1883.

6. Meierowitsch: *Centralbl. f. Bakt. (ref.)* 3:406, 1888.

7. Roger, G. H.: *Compt. rend. Soc. de biol.* 42:573, 1890.

8. Gromakowsky, D.: *Ann. Inst. Pasteur* 9:621, 1895.

9. Cobbett, L., and Melsome, W. S.: *J. Path. & Bact.* 3:39, 1896.

come with that of normal rabbits which had simultaneously been given the same quantity of streptococci. In half of the reinfected rabbits there were evidences of general immunity manifested as an early severe inflammatory reaction, which subsided in from three to four days. In a few instances, too, there were small abscesses as the end-result of the second attack of erysipelas. The second method was to inject streptococci into the peritoneal cavity and after recovery of the rabbit from this inoculation, to produce erysipelas in one ear. The course of the infection indicated that the rabbit had acquired an excellent general immunity, inferior to the local but superior to the general immunity produced by the inoculation of the opposite ear.

These investigators concluded that both local and general immunity resulted from the early inflammatory response, enhanced by the previous sensitization of the tissues. The lack of protection in the normal animals was interpreted as being due to the delay of the inflammatory reaction, which allowed the micro-organisms to multiply and produce their toxic products, which were not overcome by the phagocytes of the subsequent inflammatory reaction.

Römer<sup>10</sup> produced a local corneal immunity in the conjunctiva of one eye in rabbits by injecting small quantities of abrin. This local immunity he demonstrated by the resistance that the previously injected conjunctiva offered to further inoculation of abrin, while the opposite conjunctiva remained susceptible.

Noguchi<sup>11</sup> demonstrated a locally increased resistance to tetanus toxin in rats in which wounds were sutured with silk threads containing tetanus spores and treated with eosin, since the threads, when removed and transplanted in other rats or in the opposite sides of the same rats, caused fatal tetanus. He further showed that the leg previously resistant to the tetanus spores reacted very slowly to a subsequent inoculation of tetanus bacilli, whereas in the opposite leg or in normal rats the same quantity of bacilli produced a rapidly fatal outcome. Noguchi explained this local immunity as the result of a possible formation of antitoxins in the local connective tissues.

In 1905 Wassermann and Citron,<sup>12</sup> in testing the resistance to typhoid bacilli of animals immunized in the pleural cavity, peritoneal cavity and blood stream, noticed that the immunization was more marked if the bacilli were injected through the route previously used, and that this immunity was not dependent on the quantity of antibodies present in the blood serum. To explain this locally heightened resistance they postulated the idea of "Umstimmung," or retuning, of the local cells to an increased phagocytic activity.

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10. Römer, P.: *Arch. f. Ophth.* **52**:72, 1901.

11. Noguchi, H.: *J. Exper. Med.* **9**:291, 1907.

12. Wassermann, A., and Citron, J.: *Ztschr. f. Hyg. u. Infektionskr.* **50**:331, 1905.

Later, Wassermann and Ledermann<sup>13</sup> advised the application of killed cultures of staphylococci on infected wounds to prevent the development of complications. Similar observations were made by Levaditi,<sup>14</sup> who noticed that war wounds infected with streptococci quickly destroyed streptococci that were applied on dressings during the healing stage.

More recently, interest in the problem of local immunity has been stimulated by Besredka,<sup>15</sup> who in 1921 advanced a new theory to explain its mechanism. In this he suggested that local immunity is essentially a desensitization of certain receptive cells, which when immunized, become indifferent to specific micro-organisms, and allow them to be destroyed by the phagocytes. Although not definite as to where these receptive cells are located, he mentioned the reticulo-endothelial system as a possible location or source. He stated, furthermore, that this local immunity can be produced artificially by substances that contain the hypothetical agent, which he calls antiviral.<sup>16</sup> The application of this antiviral on or near the receptive cells produces an acquired local immunity without the intervention of antibodies. When the infection is present, this same antiviral acts as an immune serum in passive immunity, or directly produces in the tissues of the animal a medium in which the micro-organisms are incapable of multiplying, either because of desensitization of the receptive cells or because of a direct action on the bacteria present. To substantiate his ideas, the author described experimental work in animals and many clinical cases in which the antiviral had been used with favorable results.

In this country, Gay<sup>17</sup> has done much to establish local immunity as a definite entity. His work began with the production of experimental empyema in rabbits by intrapleural injections of a particular strain of streptococcus hemolyticus. He noticed under such conditions that the infection remained entirely local, although a general immunity was produced, as shown by the presence of agglutinins, opsonins and precipitins in the serum. The latter were demonstrable, however, only by a special serologic technic. Of more significance was his demonstration of the local morphologic changes produced in the pleural cavities of rabbits by the inoculation of various substances that stimulate definite cellular reactions, and the relation of these to the immunity which some of the rabbits had acquired.<sup>18</sup> From such experiments Gay concluded that the exudates containing large numbers of polymorphonuclear leukocytes were not protective when a minimal lethal dose was

13. Wassermann, A., and Ledermann, R.: *Med. Klin.* **7**:479, 1911.

14. Levaditi, C.: *Compt. rend. Soc. de biol.* **81**:1059, 1918.

15. Besredka, A.: *Ann. Inst. Pasteur* **35**:421, 1921.

16. Besredka, A.: *Bull. Inst. Pasteur* **28**:49 and 105, 1930.

17. Gay, F. P., and Stone, R. L.: *J. Infect. Dis.* **26**:265, 1920.

18. Gay, F. P., and Morrison, L. F.: *J. Infect. Dis.* **33**:338, 1923.

inoculated into the pleural cavity, whereas exudates containing larger numbers of clasmotocytes than polymorphonuclear leukocytes protected against as many as 200 M. L. D. of hemolytic streptococci. Therefore Gay concluded that the local acquired immunity was nonspecific and dependent on the high ratio of clasmotocytes to polymorphonuclear leukocytes. Later, however, he demonstrated that a more pronounced local pleural immunity could be secured when specific antibodies were added to the increased number of clasmotocytes.<sup>19</sup> Rivers and Tillett<sup>20</sup> showed also that protection against streptococci injected into the skin was far greater when the skin had been infiltrated twenty-four hours before or at the same time with the immune serum, than after similar treatment with normal serum or beef broth. Opie<sup>21</sup> observed that an antigen persisted at the site of inoculation in animals previously sensitized to that antigen, and suggested that the accumulation is due to the local presence of antibodies, which unite and fix the antigen at the site of inoculation.

Freedlander and Toomey<sup>22</sup> produced a nonspecific local immunity against *Staphylococcus aureus* in the abdominal walls of guinea-pigs, by the application of wet compresses of broth, mustard, saline solution, meat, peptone and 10 per cent horse serum. Their morphologic observations demonstrated an increased number of clasmotocytes in the subcutaneous tissues.

In summary, then, one may conclude that local immunity in varying degrees has been demonstrated in erysipelas, in experimental streptococcal empyema, during the process of healing of wounds, after non-specific stimulation with foreign proteins and other substances that bring about an inflammatory process, after injection of killed cultures of streptococci and staphylococci into local areas of the skin and after inoculation of tetanus bacilli into wounds. In all of these cases the immunity has been either a combined local and general immunity, or a general immunity manifested in a local area by a heightened, rapid inflammatory response after a nonspecific stimulation of the local tissues, or a local immunity with a general immunity of lesser degree in other portions of the body. The presence of antibodies in the blood serum has been demonstrated in many instances, and serums have been prepared that increased the local defensive powers. In explaining the mechanism of local immunity, some have postulated a defensive system that is non-specific and dependent only on the number of phagocytes; others, that the local area contains a greater concentration of antibodies, which when combined with the antigen bring about a positive chemotaxis. Practically all of the investigators of this field, however, agree that an early

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19. Gay, F. P., and Clark, A. R.: *J. Exper. Med.* **52**:95, 1930.

20. Rivers, T. M., and Tillett, W. S.: *J. Exper. Med.* **41**:185, 1925.

21. Opie, E. L.: *J. Immunol.* **8**:55, 1923.

22. Freedlander, S. O., and Toomey, J. A.: *J. Exper. Med.* **47**:663, 1928.

inflammatory reaction and an increased phagocytic activity are constantly present.

#### THE EXPERIMENTAL PROBLEM

As can be seen, the problem of local immunity is still obscure, first as to its existence, second as to its independence from a general immunity and third as to the mechanism determining it. With these considerations in mind the following questions were formulated:

1. Is there such a thing as local immunity independent from general immunity?
2. If so, what is the mechanism?
3. How can this be demonstrated experimentally?

The following experiments were undertaken in the attempt to answer these questions:

1. A histologic study of the changes in the skin of normal guinea-pigs during the process of localized intradermal injections of killed cultures of *S. aureus*.

2. A comparison of the gross reactions of the skin to inoculations of a living, virulent culture of *S. aureus* in normal guinea-pigs, in those given previous intradermal injections of 0.85 per cent salt solution and those given local intradermal injections of a staphylococcal vaccine.

3. Observation of the changes and healing processes in the skin of the abdominal wall after excision of the area inoculated with a living, virulent culture of *S. aureus* in normal guinea-pigs and in those previously treated locally with a staphylococcal vaccine.

4. A study of the histologic changes occurring at hourly intervals from the fifth to the twenty-fourth hour after intradermal injection of a living, virulent culture of *S. aureus* into the skin of the abdominal walls of normal guinea-pigs, of those previously given intradermal injections of sterile salt solution and of those previously treated intradermally with staphylococcal vaccine.

#### MATERIALS AND METHODS

In these experiments, guinea-pigs weighing from 150 to 350 Gm. were used. The material used for inoculation was from a culture of *S. aureus* obtained from a large furuncle on the upper lip of a patient who had suffered from fever, chills and malaise of ten days' duration. The virulence of the organism was determined by inoculating guinea-pigs intradermally in the abdominal wall with 0.2 cc. of a twenty-four hour agar culture, diluted in 1 cc. of physiologic solution of sodium chloride (0.85 per cent NaCl). The necrosis of an area about 0.5 cm. in diameter or the death of the guinea-pig within ninety-six hours was taken as a criterion of virulence.

Three methods of inoculation were tried to see which would cause the most pronounced inflammatory reaction: viz., with the bacteria diluted in 0.85 per cent solution of sodium chloride; with the bacteria in a 1:500 solution of calcium

chloride, and with the organisms suspended in melted agar at from 45 to 50 C. Of these three methods, the first was found easiest to control; it gave as good results as the others and did not introduce complicating reactions; it was therefore adopted as a routine procedure. A twenty-four hour agar culture of *S. aureus* was suspended in 1 cc. of sterile salt solution and heated at 60 C. for one hour, after which the suspension was transferred to a tuberculin hypodermic syringe equipped with a no. 28 gage needle, and 0.2 cc. was inoculated intradermally into the right side of the previously prepared anterior abdominal wall. An area measuring about 4 by 2 cm. had been shaved, carefully washed with tap water and soap, rinsed with sterile water and finally painted with a weak solution of iodine in a mixture of equal parts of ether and alcohol.

In all, ten daily injections, about 1 cm. apart, were made, the first three into the right anterior abdominal wall, the next four into the midline and the last three into the left abdominal wall. Control animals were similarly given injections of 0.2 cc. of 0.85 per cent solution of sodium chloride. The guinea-pigs were then left undisturbed for twenty-five days, after which they, with normal animals, were inoculated with 0.2 cc. of a twenty-four hour growth of living *S. aureus* diluted in 1 cc. of sterile salt solution.

Histologic study was made of pieces of tissue cut from the inoculated areas after they had been fastened with bamboo pegs on square frames of cork as advised by W. Bloom,<sup>23</sup> fixed in Zenker's fluid plus solution of formaldehyde, embedded in celloidin, cut at from 10 to 15 microns and stained with hematoxylin, eosin and azure II (method of Maximow<sup>24</sup>). For the study of the bacteria, Gram's and Claudius'<sup>25</sup> staining methods were used on smears and sections. For the determination of fibrin, the specific stains of Weigert,<sup>26</sup> Kockel,<sup>27</sup> Wolff,<sup>28</sup> Unna,<sup>29</sup> Schmidt<sup>30</sup> and Wallace<sup>31</sup> were used. In staining for fibrin, sections of normal and previously inoculated skins during different hours of the inflammatory process were stained, with an equal number of sections of pneumonic lung that contained fibrin. The latter served as controls on the efficiency of the stains at the time they were used.

#### GROSS AND HISTOLOGIC CHANGES PRODUCED IN THE SKIN OF NORMAL GUINEA-PIGS BY TEN DAILY INTRACUTANEOUS INJECTIONS OF STAPHYLOCOCCAL VACCINE

As a preliminary step, the effects of the daily intracutaneous injections of the heat-killed staphylococcal vaccine were first observed in twelve guinea-pigs treated as follows:

On the first day, all of the guinea-pigs were given an intradermal injection of 0.2 cc. of the vaccine. All, except the one that was to be sacrificed twenty-four

23. Bloom, W.: Personal communication.

24. Maximow, A.: *Ztschr. f. wissenschaft. Mikr.* **26**:177, 1909.

25. Claudius, M.: *Ann. Inst. Pasteur* **11**:332, 1897.

26. Weigert, C.: *Fortschr. d. Med.* **5**:228, 1887.

27. Kockel: *Centralbl. f. allg. Path. u. path. Anat.* **10**:749, 1899.

28. Wolff, E.: *Ztschr. f. wissenschaft. Mikr.* **15**:310, 1898-1899.

29. Unna, P. G.: *Monatschr. f. prakt. Dermat.* **20**:140, 1895.

30. Schmidt, H.: *Beitr. z. Anat., Physiol., Path. u. Therap. d. Ohres* **27**:455, 1929.

31. Wallace, Helene M.: *Science* **74**:369, 1931.

hours later, were vaccinated in the right upper quadrant. In the latter the injection was made in the middle of the space between the umbilicus and the lower border of the sternum. This was done to study the same histologic structure in each animal, altered only by the effects of the vaccination. Twenty-four hours later the guinea-pig vaccinated in the center of the abdominal wall was killed after the area receiving the injection had been fixed on a cork frame. The same procedure was followed in the remaining animals in order to obtain ten specimens of skin that would demonstrate the progressive daily histologic changes occurring during the process of injections.

*Gross Changes.*—The gross changes occurring in the skin of normal guinea-pigs following the first injection of the staphylococcal vaccine were either the formation of a hyperemic nodule, varying from 2 to 10 mm. in diameter, and tending to become firmer and finally disappearing but leaving a pigmented macule, or the development of a nodule that softened, ulcerated, discharged a thick, yellow purulent material and then underwent rapid healing, leaving a slightly pigmented scar.

The subsequent injections usually produced larger nodules and produced them more rapidly, with more intensive reaction at the periphery and the base of the nodule. In many instances this reaction was so violent as to produce large areas of necrosis surrounded by elevated, hyperemic and edematous borders. The nodules of the previous injections were often reactivated to an inflammatory state by the later injections, as shown by the increased size of the area of hyperemia or by the formation of a necrotic area at the point of healing or in a quiescent nodule. In many guinea-pigs after the sixth injection a sudden rapid healing occurred in the previously elevated and hyperemic nodules, the discharge of some of the pustules disappeared and the entire skin was clean. In a few instances the violent local reaction after the fourth injection resembled grossly the Arthus phenomenon in the rabbit.

*Histologic Changes.*—The first injection of vaccine produced an inflammatory reaction characterized by dilatation of the lymphatics and blood vessels, edema, diapedesis of leukocytes, hemorrhage and moderate phagocytosis of the particles of vaccine by polymorphonuclear leukocytes and a small type of mononuclear cell. During the following injections, the inflammatory reaction was more intense, and there were signs of mobilization of the undifferentiated perivascular mesenchymal cells and of the connective tissue histiocytes and other reticular cells, with the appearance of numerous actively phagocytic mononuclear cells. In the first injection, the particles of vaccine were disseminated throughout the entire section, both extracellularly and in polymorphonuclear leukocytes. In the succeeding ones, the particles tended to localize within a relatively small area, which contained many polymorphonuclear leukocytes and was surrounded by many mesenchymal cells. After the second injection, the area of greatest reaction was that of the reticular and subreticular layers, with a moderate cellular infiltration in the papillary and muscular layers.



In the reticular and subreticular layers, the predominant cells during the first injection were the polymorphonuclear leukocytes, but in the subsequent ones mononuclear cells also appeared in great numbers. The polymorphonuclear leukocytes were vacuolated, many showed pyknosis and karyorrhexis, while others were digested or were in the process of being digested by small and large mononuclear cells and elongated histiocytes.

The final result of the injections was a definite increase in the thickness of the dermis with increased proliferation of mesenchymal cells as

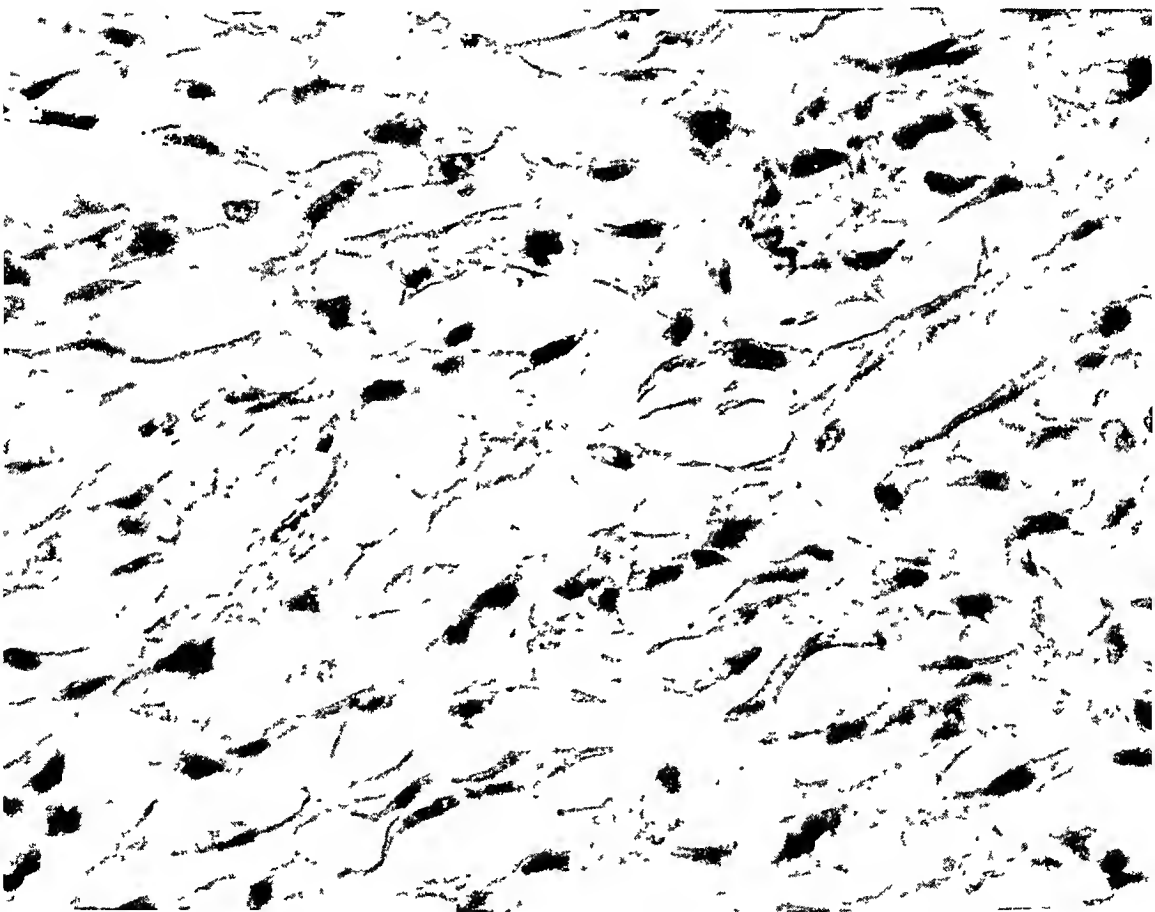


Fig 1—Photomicrograph (reduced from a magnification of  $\times 700$ ) of the subcutis of a guinea-pig after the tenth injection of the staphylococcal vaccine. Note the syncytium-like mass of mesenchymal tissue in which are many mitotic figures and some histiocytes with a foamlike cytoplasm.

manifested by the number of mitotic figures and infiltration by a great number of mesenchymal cells and leukocytes (fig. 1). The reticular and subreticular layers were greatly increased in thickness by innumerable quantities of mesenchymal and mononuclear cells, which were undergoing transformations resembling those seen in tissue cultures. There were rather numerous mitotic figures in the mesenchymal tissue and

pericytes of the blood vessels of the reticular, subreticular and muscular layers, and also a tendency toward the formation of a syncytium. Some of the mesenchymal cells were loaded with fat vacuoles. The polymorphonuclear leukocytes tended to disappear, leaving traces of their presence in the phagocytosed particles that were retained in the cytoplasm of the large mononuclear and histiocytic cells. The muscle layers were separated by a dense mesenchymal structure, which contained all the cell types of this tissue. The muscle bundles were separated by thick bundles of connective tissue. The blood vessels and lymphatics in most of the sections were dilated, patent and surrounded by many layers of undifferentiated pericytes, which frequently contained mitotic figures. In some instances the endothelial cells of the lymphatics and blood vessels were swollen. In the reticular and subreticular layers there was an increased number of new lymphatics and blood vessels. The entire thickness of the immunized skin was from three to ten times that of normal guinea-pigs.

GROSS CHANGES IN THE SKIN OF THE ABDOMINAL WALL IN  
GUINEA-PIGS AFTER INOCULATION OF LIVING,  
VIRULENT *S. AUREUS*

*Normal Guinea-Pigs.*—The gross changes produced in the skin and general health of normal guinea-pigs by the inoculation of 0.2 cc. of a living, virulent twenty-four hour agar culture of *S. aureus* were characterized, in general, by three types of reaction:

1. The formation in a few guinea-pigs of a small elevation of the skin with hyperemia and no further change during the entire period of observation.

2. The formation in some guinea-pigs of a hyperemic nodule which increased in size and density for from one to five days, until the surface became necrotic and opened to form a large, deep wound, which discharged pus and portions of the necrotic tissue for from twelve to sixteen days or more. Some of these guinea-pigs died before the wound was healed; in a few, secondary abscesses at the edges of the wound developed during the process of healing.

3. In others, an extensive cellulitis with the skin becoming purplish and edematous over nearly the entire anterior abdominal wall. The abdominal wall was cold, the guinea-pigs became extremely ill, and the majority of them died within from ten to forty-eight hours. From some of these, when the skin was cut at autopsy, much serosanguineous fluid escaped. The peritoneal surfaces were rough, with the fluid slightly turbid and increased in quantity, indicating peritonitis, presumably by extension from the area of cellulitis.

The gross effects in the twenty normal guinea-pigs inoculated with living staphylococci are summarized in table 1.

*Guinea-Pigs Previously Given Intradermal Injections of Sterile Salt Solution.*—The results obtained by the inoculation of 0.2 cc. of the living staphylococci into the guinea-pigs previously given intradermal injections of physiologic solution of sodium chloride were similar to those in the normal guinea-pigs, the gross effects being summarized in table 2.

It seems apparent, therefore, that repeated intradermal injections of salt solution did not modify in any significant way the reactivity of the tissues of the skin of the guinea-pigs to the subsequent injections of living staphylococci.

*Guinea-Pigs Previously Given Local Injections of Staphylococcal Vaccine.*—The results of the inoculation of the living staphylococci into

TABLE 1.—*Gross Effects on Normal Guinea-Pigs Inoculated Intradermally with Living S. Aureus*

Effect	Number	Per-centage
Death within ten days.....	6	30
No change.....	2	10
Complete healing after sixteen days.....	1	5
Healing wound but covered by a crust after sixteen days....	5	25
Open wound after sixteen days.....	6	30

TABLE 2.—*Gross Effects of Intradermal Inoculation of Living S. Aureus in Guinea-Pigs Previously Given Intradermal Injections of Physiologic Sodium Chloride*

Effect	Number	Per-centage
Death within ten days.....	6	30
No change.....	1	5
Complete healing after sixteen days.....	1	5
Healing wound but covered by a crust after sixteen days....	2	10
Open wound after sixteen days.....	10	50

the skin of guinea-pigs previously given local injections of the staphylococcal vaccine were as follows: In one guinea-pig a small, firm nodule appeared, which lasted for a few days and then gradually regressed. In others, small pustules developed, which discharged a thick, yellow purulent material and healed in from seven to fourteen days, leaving slightly pigmented scars. In most of the animals, however, nodules developed which increased in size and ulcerated after from three to five days, forming wounds that healed with small scars in fifteen days or less. It was apparent that these reacted quickly and effectively with rapid healing; furthermore, the death rate was one-half that of the normal guinea-pigs and that of the guinea-pigs previously inoculated with sterile physiologic solution of sodium chloride. The results for this group of twenty animals are summarized in table 3.

These experiments demonstrated that there is a great variability in the reactivity of the skin of normal guinea-pigs to the inoculation of living *S. aureus*. This may be explained by variation in natural immunity, which is more marked in some animals than in others. The results in the guinea-pigs given previous local injections of the staphylococcal vaccine indicated that the injection of killed staphylococcal vaccine had produced a change in the reactivity of the inoculated tissues to the extent of preserving the life of some guinea-pigs and of accelerating the healing process and restricting the dissemination of the bacteria in others.

TABLE 3.—*Gross Effects of Intradermal Inoculation of Living S. Aureus into Guinea-Pigs Previously Given Intradermal Injections of Staphylococcal Vaccine*

Effect	Number	Per-centage
Death within ten days.....	3	15
No change.....	1	5
Complete healing after fourteen days.....	14	70
Healing wound but covered by a crust after sixteen days....	1	5
Open wound after sixteen days.....	1	5

TABLE 4.—*End-Results of Inoculation of Living S. Aureus in Normal Guinea-Pigs and Those Previously Given Injections of Staphylococcal Vaccine from Which Inoculated Areas Were Excised*

Normal			Vaccinated		
	Num-ber	Percent-age		Num-ber	Percent-age
Dead .....	4	40	Dead .....	0	0
Healed in 13 days.....	1	10	Healed in 8 days.....	2	20
Healed in 14 days.....	2	20	Healed in 9 days.....	3	30
Healed in 19 days.....	3	30	Healed in 12 days.....	5	50

#### EFFECTS OF EXCISION OF THE AREA OF INOCULATION AFTER INTRADERMAL INJECTION OF LIVING STAPHYLOCOCCI

The rapidity of dissemination of living bacteria from the inoculated area was tested in another series of twenty guinea-pigs, ten of which were normal and ten of which had been previously treated locally with the staphylococcal vaccine. The guinea-pigs were paired according to weight, and the inoculations were performed five minutes apart. At intervals of one, two, three, five, seven, nine, twelve, fifteen, eighteen and twenty-four hours, a pair of animals was anesthetized, and from each a piece of tissue 10 by 10 by 2 mm. was excised around the point of inoculation. The wounds were closed with two or three stitches of silk no. 2, and the gross changes subsequently determined. Table 4 summarizes the after-effects. In the normal animals, healing was slow;

all the wounds opened within from one to four days, and there was a purulent discharge for more than sixteen days. The area where the silk stitches were placed became necrotic, and large pieces of greenish tissue sloughed off. The fatality rate in this series of animals was 40 per cent. The healing time was between thirteen and nineteen days, and in many guinea-pigs secondary abscesses developed in the neighborhood of the wound.

In contrast to these findings, the healing of the wounds in the guinea-pigs previously given injections of the staphylococcal vaccine was rapid, in some instances the wounds remaining closed and healing by first intention. In others, although the wounds opened completely, healing occurred within from eight to twelve days. Furthermore, no deaths occurred in these animals, and no abscesses recurred.

These experiments demonstrated that the bacteria in the skin of normal guinea-pigs disseminated quickly through the surrounding tissue, so that the excision of the infected area at one hour or at twenty-four hours did not prevent the fatal outcome or prevent delay in the healing processes. In the guinea-pigs previously given injections of the staphylococcal vaccine the bacteria were considerably localized, as demonstrated by the fact that the removal of the infected area protected the life of 40 per cent of these guinea-pigs, and shortened the period of healing of the wounds. Furthermore, the rapidity of healing of these tissues by primary union was striking, indicating that the harmful effects of the staphylococci had been largely eliminated.

#### HISTOLOGIC CHANGES AFTER INTRADERMAL INOCULATION OF LIVING *S. AUREUS* IN THE ABDOMINAL WALL IN GUINEA-PIGS

Similar experiments were next performed in order to study histologically at hourly intervals after the fifth hour the changes produced in the abdominal wall following the intradermal inoculation of 0.2 cc. of a living twenty-four hour agar culture of *S. aureus*. Sixty guinea-pigs were used, twenty normal, twenty previously given intradermal injections of the sterile salt solution, and twenty locally inoculated in the abdominal wall with the staphylococcal vaccine. From these three sets of guinea-pigs, groups of three of approximately equal weight (one from each set) were selected. In each group the individual inoculations of the living staphylococci were done five minutes apart in order to allow time to fix the tissues when they were later removed, and yet have similar hourly periods. After the fifth hour a group of three guinea-pigs was sacrificed each hour for the next twenty-four hours. By this procedure tissues showing hourly histologic changes following the inoculation of living staphylococci were obtained from twenty groups of guinea-pigs.

The general histologic changes occurring under these conditions have been described and illustrated in a previous paper (Cannon and

Pacheco), where the histologic differences in the normal and immunized animals were demonstrated. In the former, the characteristic picture was an extensive edema of the skin with dilatation of the blood vessels and lymphatics, hemorrhage and moderate infiltration by granular leukocytes and by a lesser number of small mononuclear cells. Histiocytes were inconspicuous, and there was no evidence of activation of the mesenchymal tissues. Some of the muscle bundles were undergoing hyaline degeneration. The blood vessels were dilated and engorged with blood, and at times contained many leukocytes that were in active stages

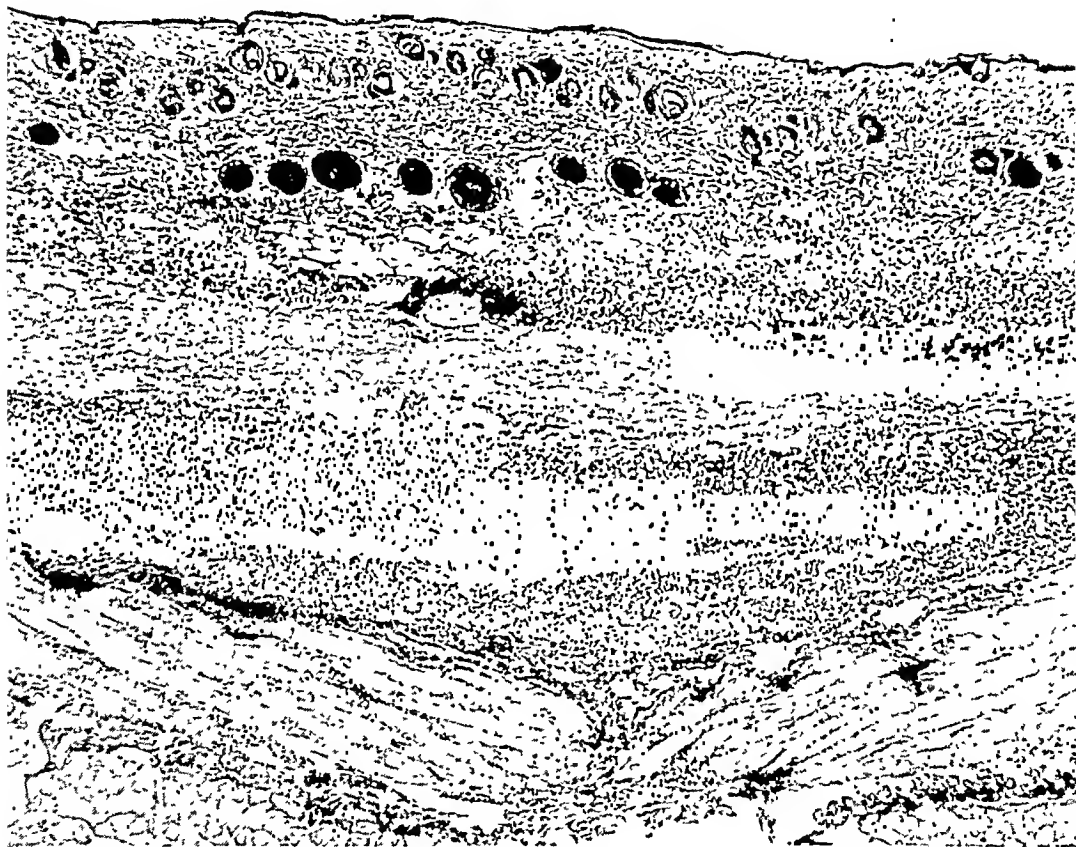


Fig. 2.—Photomicrograph (reduced from a magnification of  $\times 70$ ) of a section through the skin and entire abdominal wall of a normal guinea-pig inoculated with 0.2 cc. of a suspension of living, virulent *S. aureus*. The tissue was excised six hours after inoculation. The bacteria are disseminating through the reticulin or collagenic fibrils of the subreticular layer. There is a moderate cellular response, most of the cells being polymorphonuclear leukocytes, which contain many phagocytosed bacteria. The black area around the large venule consists of a concentrated mass of extracellular staphylococci, and the edematous layer of reticular fibrils beneath this contains large numbers of extracellular bacteria as shown in figure 3. The infection is obviously generalized, in spite of the abundant polymorphonuclear leukocytic response.

of diapedesis. The majority of the lymphatics were patent and dilated. No fibrin was found by the use of specific stains. The polymorphonuclear leukocytes showed innumerable bizarre figures, pyknosis, karyorrhexis and vacuoles. Enormous numbers of the polymorphonuclear leukocytes contained ingested staphylococci; many of the microorganisms were disseminated in long rows through the entire section, even extending into the connective tissue septums of the muscle layers. In many instances there were extensive areas of necrosis in the epi-



Fig. 3.—Photomicrograph (reduced from a magnification of  $\times 2,650$ ) of the section shown in figure 2. The bacteria are disseminating through the collagenic or reticulin fibrils. The cellular reaction is slight in this area. Note the absence of any agglomerative tendency of the bacteria.

dermis, papillary layers and muscularis. The subreticular layer was filled with polymorphonuclear leukocytes, eosin-stained precipitate and isolated groups or rows of free bacteria (fig. 2). The picture as a whole was that of an extensive cellulitis. It is noteworthy that in spite of widespread phagocytosis by the polymorphonuclear leukocytes the infection was disseminating widely.

The histologic changes in the skin of guinea-pigs previously given intradermal injections of normal salt solution were practically indistinguishable from those of the normal animals and will not be described in detail.

The histologic changes in the skin of guinea-pigs previously given local injections of the staphylococcal vaccine were characterized by a marked tendency to localization and clumping of the bacteria in a small area, which was filled with polymorphonuclear leukocytes, red blood

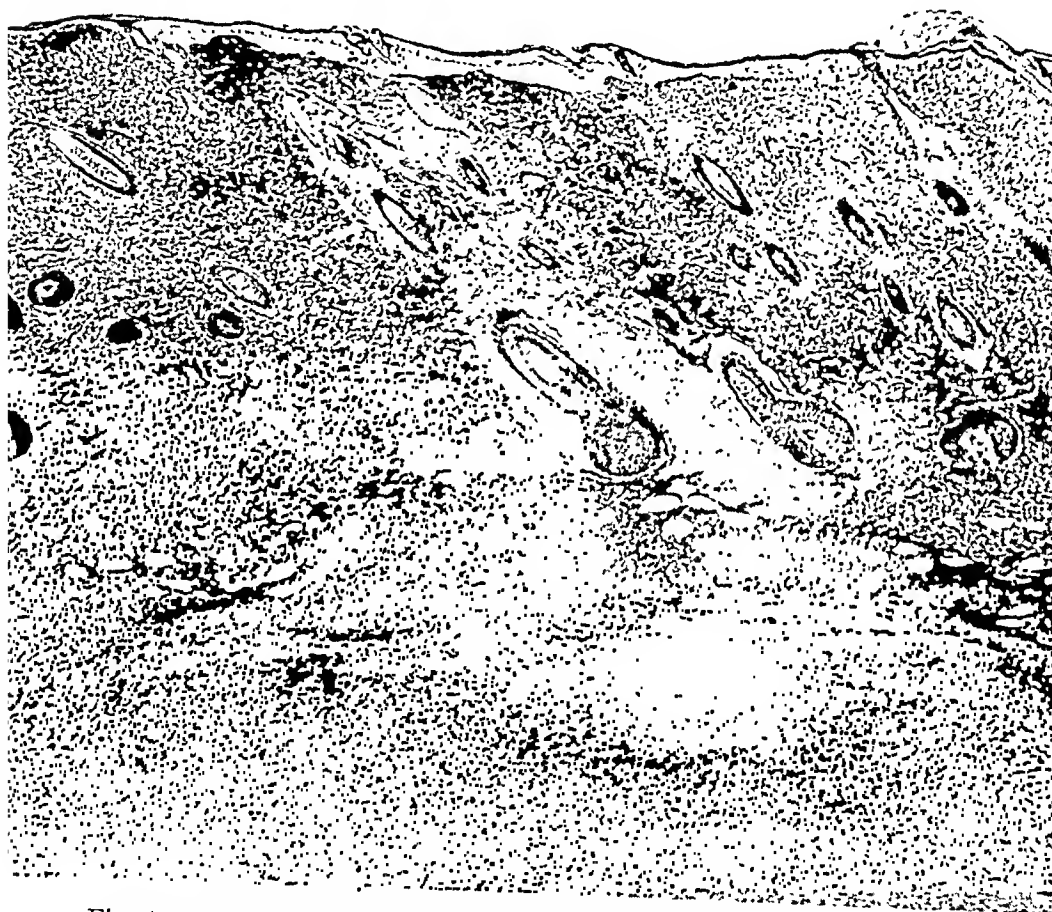


Fig. 4.—Photomicrograph (reduced from a magnification of  $\times 70$ ) of a section through the skin and a portion of the subreticular zone of a guinea-pig's abdominal wall previously immunized locally with the staphylococcal vaccine and later inoculated with 0.2 cc. of a living culture of *S. aureus*. Section taken six hours later. Note the localization of the bacteria in large masses in a small area, which is surrounded by a dense layer of mesenchymal cells. The area of polymorphonuclear infiltration and edema is very small. The black areas at the margins of the area of edema are masses of extracellular bacteria as shown in figure 5.

cells and small mononuclears (fig. 4). This localized area was in turn surrounded by large mononuclear leukocytes, histiocytes and many undifferentiated mesenchymal cells. As early as six hours, extracellular



masses of bacteria, as well as intensive phagocytosis by the small and large mononuclear and polymorphonuclear leukocytes, were noted. The polymorphonuclear response was intensive, but was localized largely to the area where the bacteria were fixed. Outside of the area, fewer polymorphonuclear leukocytes could be found between the mesenchymal tissues. The hemorrhage was also localized to the area where bacteria and the polymorphonuclear leukocytes were gathered, with the exception of two cases in which hemorrhage had occurred into the surrounding mes-

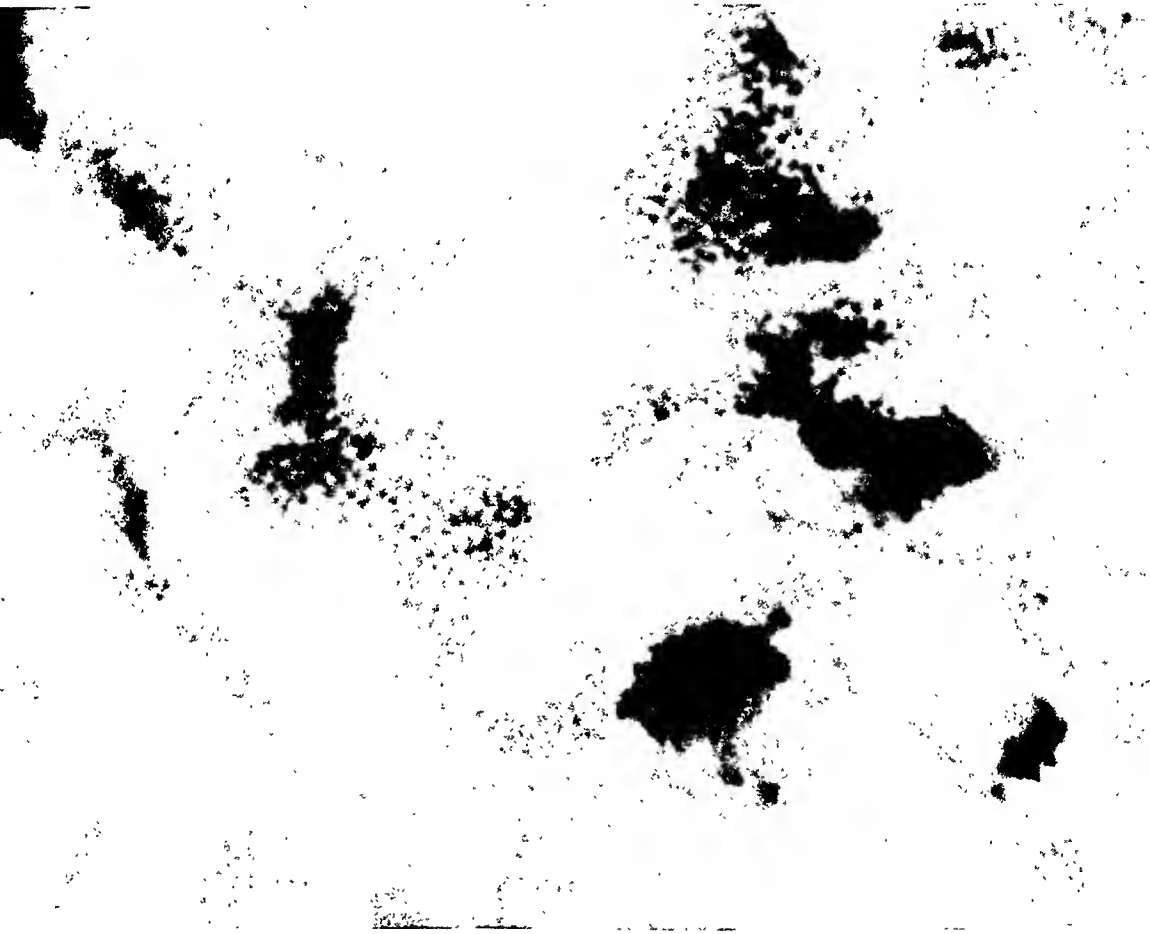


Fig. 5.—Photomicrograph (reduced from a magnification of  $\times 2,650$ ) of the section shown in figure 4 in an area where the bacteria are less concentrated. The bacteria are in masses. In many places the outlines of the bacteria cannot be made out.

enchymal tissue and the muscle layers. From the fifth to the twenty-fourth hour the polymorphonuclear leukocytes showed numerous bizarre shapes, were poorly stained, were vacuolated and showed evidences of degenerative changes. The mononuclear cells surrounding the polymorphonuclear leukocytes were in active stages of phagocytosis, as shown by the numbers of bacteria and polymorphonuclear leukocytes within their cytoplasm.

The bacteria as a whole were localized in large clumps to a small area as early as six hours after the inoculation (fig. 5), and after twelve hours most of them were intracellular. Both intracellular and extracellular injury of bacteria was indicated by their swollen and poorly stained appearance.

In the remainder of the reticular and subreticular layers there were several rows of connective tissue cells, many of which were proliferating, as shown by numerous mitotic figures. The area of localization was, as a rule, in the reticular and subreticular layers, although in a few cases the bacteria were localized in the papillary layer. In others there was a local necrosis of the epithelium and papillary layers. The papillary and muscular layers contained local phagocytic cells of many bizarre shapes, as though they had been fixed while in an active stage of ameboid movement, but very little necrosis was noted in these layers. The blood vessels and lymphatics were largely patent, and in many instances there were many patent new small blood vessels in the mesenchymal tissue of the subreticular layer. After a thorough search few thrombosed blood vessels or lymphatics were found; on the other hand, many of these structures seemed to be dilated. Fibrin could not be found with specific stains, and with hematoxylin and eosin the picture was that of a compact mass of bacteria, polymorphonuclear leukocytes and mononuclear cells, surrounded by a dense mass of mesenchymal tissue in which Mallory's connective tissue stain revealed a network of reticulin and collagenic fibrils in the reticular and subreticular layers.

#### COMMENT

These experiments demonstrate that living virulent staphylococci may be effectively localized in the cutaneous tissues of guinea-pigs previously given injections in the same area of a heat-killed suspension of *S. aureus*. This vaccination leads to the development of a locally superior mechanism for the disposal of living staphylococci when they are injected later. The experiments, however, do not differentiate between the effects of local and general resistance. Data concerning that problem will be presented in a later paper.

The specific purpose of this work was to study in detail the morphologic changes induced in the mesenchymal tissue by the injection of a staphylococcal vaccine, and to determine to what extent these changes altered the normal reactivity to a living, virulent suspension of *S. aureus*. Consequently, serologic studies of the blood serum and local tissues were not made.

It is apparent that the intradermal injection of heat-killed staphylococci called forth a pronounced cellular response in the skin of the anterior abdominal wall of guinea-pigs. This response was predominantly of polymorphonuclear leukocytes, followed shortly by mobilization

and proliferation of mononuclear cells. The latter arose *in situ* to a large extent, as shown by the numerous mitotic figures, but many also entered from the blood stream. By the fourth day these mononuclears were actively phagocytic and contained in their cytoplasm particles of vaccine and polymorphonuclear leukocytes in various stages of disintegration. By the tenth day of the inoculations the dermis was from three to ten times the thickness of that in normal animals. The reticular and subreticular layers looked like a syncytium, in which were many new blood vessels and lymphatics. The picture in general resembled that described by Gay, Clark and Linton <sup>32</sup> in the pleural and peritoneal wall of rabbits into which nonspecific irritants, such as aleuronat-starch mixtures, had been injected.

The inoculation of living, virulent staphylococci into the abdominal wall of guinea-pigs previously treated with the staphylococcal vaccine was followed by a distinctly different response from that of normal guinea-pigs, as measured by the degree of illness, the fatality rate, the ability of the tissues to heal, the histologic reaction and the degree of phagocytosis. The degree of illness was never as pronounced in the previously treated guinea-pigs as in the normal ones. The fatality rate was one-half that of the normal guinea-pigs, and the ability of the tissues to heal after an area of necrosis was produced or after the excision of the area inoculated was surprisingly rapid and without complications. The histologic reaction was characterized by a localization of the staphylococci in clumps, usually in a small area of the subreticular layer, although in two instances this localization took place in the papillary layer. The epithelial covering of the area of localized cocci usually became necrotic. The central portion of the cellular reaction consisted of polymorphonuclear leukocytes and a few mononuclear cells. These polymorphonuclear and mononuclear phagocytes in turn were localized by innumerable mesenchymal cells of the local tissues. The phagocytic activity of the cells was marked, as evidenced by the great number of cocci within a single phagocyte.

The mechanism of localization of such large numbers of virulent staphylococci within a small area of skin of a locally treated guinea-pig as contrasted with their widespread dissemination in the skin of a normal guinea-pig, is obviously of great significance. Various ideas have been advanced to explain this mechanism. Cobbett and Melsome <sup>9</sup> thought that it was the result of an early inflammatory reaction, which destroyed the micro-organisms before they had time to adjust themselves to the new environment, to proliferate and to liberate their toxins. Wassermann and Citron <sup>33</sup> postulated the idea of "Umstimmung," or retuning,

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32. Gay, F. P.; Clark, A. R., and Linton, R. W.: Arch. Path. **1**:857, 1926.

33. Wassermann, A., and Citron, J.: Deutsche med. Wchnschr. **31**:573, 1905.

of the cells of the area which had been sensitized. Noguchi<sup>31</sup> mentioned the possibility of a local formation of antibodies in the connective tissue of the areas previously in contact with a bacterial antigen. Opie<sup>34</sup> demonstrated the fixation of an antigen at the site of inoculation in tissues sensitized to that antigen. His explanation of such a phenomenon was based on the assumption of the local occurrence of an anaphylactic inflammation.

Menkin<sup>35</sup> recently advanced the idea that bacteria and particulate matter are fixed in an area previously inflamed because of the barrier action of thrombosed lymphatics and the deposition of fibrin. This explanation, however, seems inadequate in the present series of experiments, first, because the sections show many more patent than thrombosed blood vessels and lymphatics, and secondly, because of a complete absence of fibrin as revealed by the use of specific staining methods. Although there is a semblance of fibrin in sections of the skin of normal guinea-pigs inoculated with staphylococci and stained with hematoxylin and eosin, such is not the case in sections of the skin of guinea-pigs previously treated with the staphylococcal vaccine. Furthermore the use of nine specific stains for fibrin has failed in every instance to demonstrate its presence.

The countless numbers of phagocytes may act as a mechanical barrier, especially in cases of nonspecific immunity, but in specific immunity the local presence of immune bodies must be taken into consideration. It is probable that the general mechanism of fixation is as follows: The vaccine introduced intradermally is retained and metabolized principally by the local phagocytes, with the resulting formation of antibodies which in turn tend to fix the antigen at the site of inoculation by a precipitating reaction, as Opie<sup>4</sup> demonstrated, or else the union of antigen with antibody produces a physicochemical change in the tissues that hinders dissemination of the antigen from the area. Evidence for the presence of agglomerating or flocculating antibodies is furnished by the morphologic demonstration of agglutination in vivo, as reported in a previous paper by Cannon and Pacheco.<sup>36</sup> Further evidence of the presence of local antibodies is suggested by the great degree of swelling of the bacteria injected into the locally treated tissues and by the vigorous phagocytosis by the microphages and macrophages. Similar observations were made by Bordet<sup>37</sup> and Tsuda.<sup>38</sup> More recently, Cannon and Sullivan<sup>39</sup> demonstrated by methods of chemical extraction that anti-

34. Opie, E. L.: *J. Immunol.* **9**:255, 1924.

35. Menkin, V.: *J. Exper. Med.* **53**:171, 1931.

36. Cannon, P. R., and Pacheco, G. A.: *Am. J. Path.* **6**:749, 1930.

37. Bordet, J.: *Ann. Soc. roy. d. sc. méd. et nat. de Brux.* **4**:455, 1895.

38. Tsuda, S.: *Virchows Arch. f. path. Anat.* **247**:121, 1923.

39. Cannon, P. R., and Sullivan, F. L.: *Proc. Soc. Exper. Biol. & Med.* **29**:517, 1932.

bodies may be formed locally in an area of local immunization and may persist there in greater concentration than elsewhere, as, for instance, in adjacent unimmunized skin, blood serum, spleen and liver. These findings strengthen the view that local formation and concentration of antibodies may play an important part in the mechanism of local immunity. The "Umstimmung," or retuning, as suggested by Wassermann and Citron<sup>33</sup> may thus be explained as due to the local concentration of immune bodies formed by locally mobilized mesenchymal cells, the latter having elaborated the immune bodies during the period of local immunization. The introduction of antigen into a tissue containing locally a high concentration of specific antibodies between and probably on the surfaces of histiocytes may lead to an antigen-antibody reaction tending to localize the antigen immediately. Secondary to this union, the vasomotor reactions and leukocytic infiltration tend further to keep the antigen fixed, thus preventing the harmful effects of its generalization.

#### CONCLUSIONS

The continued local intradermal treatment of guinea-pigs with a heat-killed suspension of *S. aureus* leads to the proliferation and mobilization of large numbers of macrophages in the area treated.

The subsequent inoculation into such an area of living, virulent staphylococci is followed by an accelerated inflammatory response and healing, with localization of the bacteria in the area inoculated.

The localization is due primarily to an antigen-antibody reaction whereby the bacteria tend to become agglomerated and presumably opsonized, and then engulfed by phagocytes, both microphages and macrophages.

This localization does not seem to be significantly influenced by such mechanical barriers as a deposition of fibrin or thrombosis of lymphatics and blood vessels.

As a result of these local tissue changes the harmful effects of the generalization of the bacteria are to a great extent prevented.

# THE STEM CELL OF THE MONOCYTE

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BOSTON

In a recent communication,<sup>1</sup> I described a method of silver impregnation of blood cells spread on cover glass films that, owing probably to dissociation, concentration and partial loss of hemoglobin, revealed an unusual and probably artificial structure in the erythrocyte and gave unusually sharp details of the nuclei of white blood cells. It is believed that considerably more precision in outlining chromatin is achieved with this than with the usual technical procedures.

With this method at hand, a case of monocytic leukemia gave an opportunity to study the nuclear structure of the monocyte in detail.<sup>2</sup> The case was of particular value for this type of study, as the cells in the circulating blood belonged almost exclusively to the monocytic series, and at certain periods of the illness cells at all stages of development were present. A brief outline of the history of the case follows:

A white man, aged 30, who was a poultry expert, entered the hospital with the complaints of weakness, soreness of the gums and painful swelling in the left side of his neck and face. He had noticed undue fatigability for one and one-half years, which had become more severe during the past few weeks. Two weeks before he entered the hospital, his gums had become sore, and the lower teeth had become loose. Following this, sore throat and the painful swelling in the left side of the neck had developed.

Physical examination revealed a pale young man with a swelling at the left side of the face and neck and small purpuric spots scattered over the trunk and lower extremities. The gums were soft and discolored, with a small area of ulceration at the margin. The tonsils were enlarged and reddened. There were moderately enlarged lymph nodes in the submental and upper cervical regions, and several nodes from 2 to 3 cm. in diameter in both axillae. The inguinal nodes were small. The edge of the spleen was felt 4 cm. below the costal margin.

In spite of repeated transfusions of blood and other therapeutic measures, the course of the illness was rapidly unfavorable, with death eighteen days after entry.

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Submitted for publication, Jan. 30, 1932.

From the Thorndike Memorial Laboratory and the Second and Fourth Medical Services (Harvard) of the Boston City Hospital, and the Cancer Commission of Harvard University.

1. Rinehart, J. F.: Unusual Structures in the Erythrocyte: II. A Precise Nuclear Impregnation Method, *Anat. Rec.* **52**:151, 1932.

2. Dr. George R. Minot and Dr. Claude E. Forkner gave me permission to study the blood in this case.

The majority of the cells in the blood throughout the course of the illness were monocytes, typical in reaction in supravitality stained and Wright-stained preparations.

All of the differential counts of white blood cells given in the following paragraphs were made from supravitality stained preparations by an independent observer experienced in the use of this method.<sup>3</sup>

The blood on entry showed: red blood cells, 2,190,000 per cubic millimeter; hemoglobin, 8 Gm. per hundred cubic centimeters; white blood cells, 62,000 per cubic millimeter, with the following differential count—mature monocytes, 76.5 per cent; young monocytes, 12.5 per cent; monoblasts, 5 per cent; polymorphonuclear neutrophils, 0.5 per cent; polymorphonuclear basophils, 0.5 per cent; myelocytes, 0.5 per cent, and small lymphocytes, 4.5 per cent.

During the course of the illness, the total number of white blood cells ranged from 34,000 to 150,000 per cubic millimeter, with mature monocytes ranging in number from 72 to 96 per cent, young monocytes from 2 to 20 per cent and monoblasts from 1 to 5 per cent. Histiocytes (macrophages) were not seen.

#### BRIEF REVIEW OF THE LITERATURE ON THE HEMOHISTIOBLAST

Because of its important rôle in this study, I shall briefly review the history and present status of the cell known as the hemohistioblast or the Ferrata cell. While no attempt is made to review completely the extensive literature bearing on the cell, reference is made to representative pertinent data.

Franco and Ferrata,<sup>4</sup> in 1919, described in the peripheral blood and spleens of patients with chronic myelogenous leukemia, a cell of distinctive morphologic character that had not been previously recognized. Subsequently, Ferrata<sup>5</sup> and others advanced a convincing number of data supporting the separate identity of this cell, which Ferrata called the hemohistioblast. This investigator identified the cell chiefly by the unique character of its nucleus, which he likened to a sponge. The cytoplasmic outline is usually irregular and frequently shows pseudopodia. Ferrata's conception of this cell is briefly as follows: It is the stem cell from which the monocyte and the lymphocyte arise, and from which cells of the granulocytic series may be derived without passing through the myeloblastic stage. The cells are divided into three categories as follows: (1) cells without granules, (2) cells with azure granules or filaments and (3) cells with specific neutrophilic or eosinophilic granules. In the cells of the first two groups, from one to several nucleoli are present.

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3. The differential counts were made by Dr. C. E. Forkner. He also permitted me to use his records and gave other help in this study. A more detailed report of this case will be made by Dr. Forkner at a later date. Miss Charlotte Clarke and Miss Rose Hermann prepared the excellent cover glass blood films used in this study.

4. Franco, E., and Ferrata, A.: *Arch. per le sc. med.* **43**:109, 1919.

5. Ferrata, A.: *Haematologica* **2**:242, 1921; **5**:228, 1924.

Gasbarrini<sup>6</sup> noted the presence of the hemohistioblast in lymphatic leukemia. Reitano<sup>7</sup> recorded observations on the hemohistioblast in monocytic leukemia. Di Guglielmo<sup>8</sup> presented evidence that the primitive cell of the circulating blood in the embryo in the prehepatic period of the formation of the blood was the hemohistioblast. Esposito<sup>9</sup> confirmed Ferrata's observations on this cell in five cases of myelogenous leukemia and observed a similar cell in three cases of lymphatic leukemia. Massazza<sup>10</sup> recorded the presence of the hemohistioblast in the blood of a patient with severe anemia during pregnancy. De Souza Aranha<sup>11</sup> observed, in the blood of a person with malaria and syphilis, true hemohistioblasts of the type recorded for leukemia, coincident with monocytosis. Richter<sup>12</sup> reported observations on the hemohistioblast in four cases of myelogenous leukemia, one case of lymphatic leukemia and one case each of malaria, staphylococcus septicemia and von Jaksch's anemia. Fontana,<sup>13</sup> in a study of an unusual clinical syndrome described as "mielosi eritremica pseudo-aplastica," placed the hemohistioblast as the precursor of the megaloblast.

The identity of the cell has, however, been contested by several hematologists. Naegeli,<sup>14</sup> Ringoen,<sup>15</sup> Lambin<sup>16</sup> and Fegler<sup>17</sup> considered the cell to represent damaged forms of the usual circulating elements. Lambin and Ringoen offered as a serious argument against the identity of the cell the failure to see the cell in vitally stained preparations. I do not consider this a valid argument for the following reason: The hemohistioblast is recognized chiefly by its peculiar open, lacelike or spongelike nucleus, and the supravital preparation is of little value in studying the minutiae of the internal architecture of the nucleus. Further, in cases of leukemia there are cells, particularly the more primitive forms, that cannot be identified by even the most experienced observer. There are, of course, damaged cells in almost any preparation of blood containing many young white blood cells. Some of these broken cells have a certain resemblance to the hemohistioblast, but I believe that this resemblance is superficial. The hemohistioblast presents

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6. Gasbarrini, A.: *Haematologica* **1**:260, 1920.

7. Reitano, D.: *Haematologica* **3**:524, 1922.

8. di Guglielmo, G.: *Haematologica* **3**:469, 1922.

9. Esposito, A.: *Haematologica* **4**:269, 1923.

10. Massazza, M.: *Haematologica* **6**:94, 1925.

11. de Souza Aranha, M. E.: *Haematologica* **6**:328, 1925.

12. Richter, M. N.: *Am. J. M. Sc.* **169**:336, 1925.

13. Fontana, A.: *Haematologica* **10**:303, 1929.

14. Naegeli, O.: *Blutkrankheiten und Blutdiagnostik*, ed. 4, Berlin, Julius Springer, 1923.

15. Ringoen, A. R.: *Folia haemat.* **33**:149, 1927.

16. Lambin, Paul: *Haematologica* **8**:1, 1927.

17. Fegler, G.: *Compt. rend. Soc. de biol.* **96**:347, 1927.



a nuclear structure too delicate and too clearly delineated to be considered a damaged element. A further objection of Lambin and Ringoen to the identity of the cell is the fact that it does not resemble, in nuclear structure, the resting cell of the reticulo-endothelium or its derivative, the phagocytic histiocyte (macrophage). Ferrata recognized this. I agree that the histiocyte and the hemohistioblast present different nuclear patterns. The histiocyte possesses a distinctly denser, more closely knit chromatin than the hemohistioblast. (Contrast the histiocyte in figure 1 with the hemohistioblasts in figures 2 and 3). Ferrata considered, however, that the hemohistioblast is derived from the reticulo-endothelium, and that the transformation to a cell with the spongy-

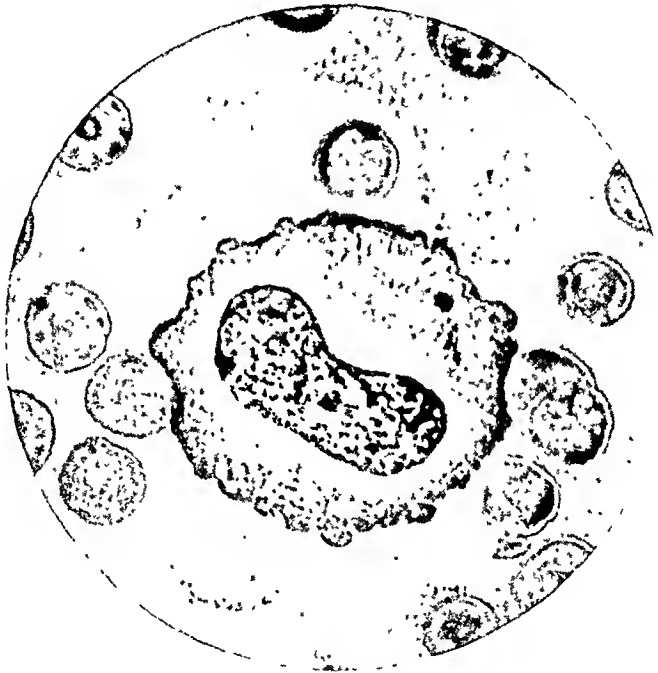


Fig. 1.—A macrophage (histiocyte) showing the relatively dense, closely drawn nucleus and the large amount of cytoplasm that are characteristic of this cell. The cytoplasm contains a phagocytosed fragment of an erythrocyte. Note that this cell differs in nuclear character from the hemohistioblast. Photomicrograph;  $\times 1,750$ .

appearing nucleus is a morphologic expression of the mobilization of the cell. I believe it is probable that, under a stimulus causing active proliferation of the cells of the reticuloendothelium, they assume a nuclear form of more primitive type with a looser organization of chromatin. This opinion finds support in the observations of Merklen and Wolf on the pathologic changes in monocytic leukemia, in which they found the essential abnormality to be a proliferation of the specific or reticulo-endothelium. They described an enlargement of the reticulum cells with "brightening" of the nucleus and loosening of the chromatin into a fine network.

One observation by Ferrata<sup>5</sup> of fundamental significance and apparently frequently overlooked is that a close similarity in nuclear pattern obtains between the hemohistioblast and the cell of the embryonic mesenchyme. It is generally considered that in the adult organism cells exist that are of mesenchymal type of potentiality. It would not, then, seem unusual in the presence of a stimulus, as in leukemia, occasioning active proliferation of mesenchymal cells or of a tissue little differentiated from the mesenchyme, as the reticulo-endothelium, to have cells of mesenchymal form produced and appearing in the circulating blood. While one may object, perhaps, to the complete thesis

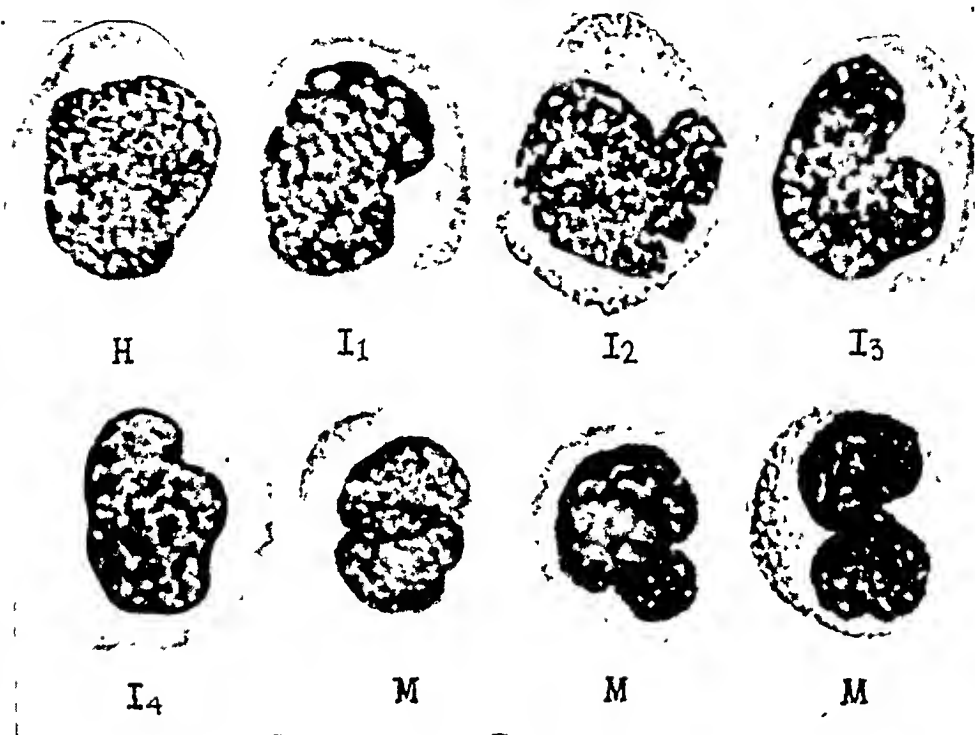


Fig. 2.—Cells selected from a single blood film in a case of monocytic leukemia illustrating the spongelike nuclear character of the hemohistioblast (*H*) and showing intermediate stages of differentiation (*I*<sub>1</sub> to *I*<sub>4</sub>) into the mature monocytes (*M*). Photomicrograph;  $\times 1,750$ .

of Ferrata, there appears to be a strong chain of evidence supporting the separate identity and great potentialities of this cell. To recapitulate, the hemohistioblast is found almost consistently in myelogenous leukemia and has been described in lymphatic leukemia and in monocytic leukemia; the cell has been shown to be the circulating stem cell in the prehepatic period of the formation of the blood in the embryo, and the megaloblast has been traced to the same cell. Further, the cell, as Ferrata pointed out, corresponds in nuclear type to the mesenchymal cell, the logical form of a multipotential element.

It is the purpose of the subsequent section to trace the nuclear development of the monocyte from a cell of this type in monocytic leukemia.

In this study, there was observed a small but definite number of cells corresponding in nuclear architecture to the hemohistioblast. Further, many cells were seen with a nuclear structure intermediate in

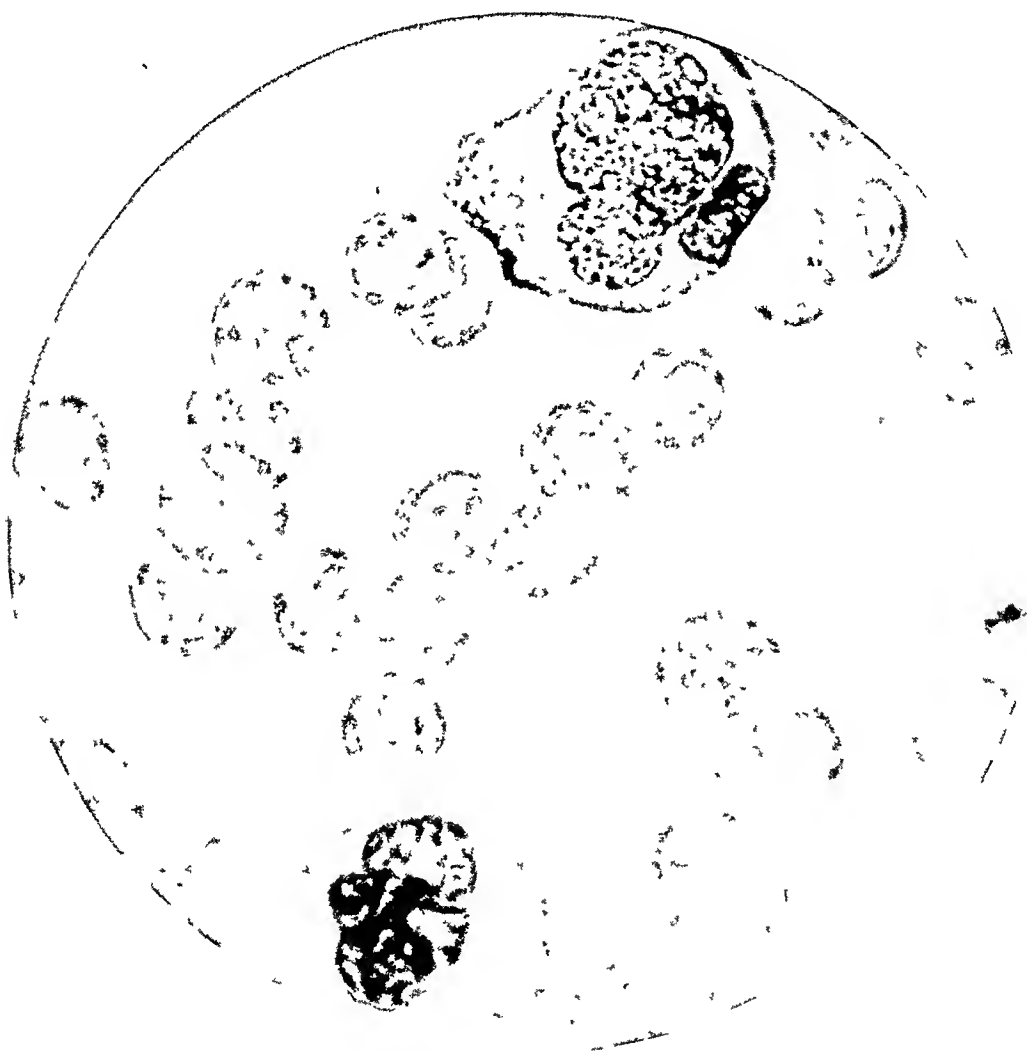


Fig 3—The cell above shows the typical sponge-like nuclear character of the hemohistioblast, and the cell below, the softly drawn, skelmlike nucleus of the mature monocyte Photomicrograph,  $\times 1,750$

form between the hemohistioblast and the monocyte. It has been possible, from a single blood film, to select a series of cells showing a gradual transformation of the nuclear structure from that of the hemohistioblast to that of the mature monocyte (fig. 2).

A brief description of the nuclear architecture in the various forms, as revealed by my technic, is given in the following paragraphs.

## DEVELOPMENT OF THE MONOCYTE FROM THE HEMOHISTIOBLAST

*The Hemohistioblast.*—In dry fixed films stained by the silver impregnation method, the nucleus of the hemohistioblast presents a net-like structure. The interlacing of the fine, but clearly separated, chromatin filaments produces a delicate lacelike pattern. From two to five small, round or oval nucleoli are usually visible in addition to the tiny open spaces between the lines of chromatin. The cytoplasmic outlines frequently are indistinct and may be quite irregular. Figure 3 shows a hemohistioblast with this characteristic architecture, accompanied by an adult monocyte. The hemohistioblast shown in figure 3 has features that make the supposition that it is a traumatized cell very unlikely. The nucleus maintains its normal rounded contour, and an unusual accessory lobule is undisturbed. Further evidence against trauma causing the appearance of hemohistioblasts is the fact that in traumatized portions of films the injured monocytes were recognized as such and did not show the nuclear structure of the hemohistioblast or the intermediate forms to be described. The cell *H* in figure 2 again illustrates the characteristic nuclear pattern of the hemohistioblast.

*Intermediate Forms.*—Cells intermediate in nuclear form between the hemohistioblast and the monocyte are best described by reference to figure 2. It will be seen that the intermediate forms, while retaining to some degree the spongelike nuclear character of the hemohistioblast, show thickening and apparent fusion of the filaments of chromatin, accompanied by indentation of the nucleus as the adult type of cell is approached. See figure 2 *I*<sub>1</sub> to *I*<sub>4</sub>.

*The Mature Monocyte.*—The majority of the cells present were mature monocytes. The mature monocyte stained by the silver impregnation method presents an aspect quite characteristic and is rather clearly differentiated from the other leukocytes. The chromatin presents a moderately dense, but dull, softly drawn appearance, different from the denser, more uniform chromatin of the lymphocyte, and the brighter, heavy lines or blocks of chromatin in the myelocyte. The nuclei, of course, show the characteristic foldings, producing a series of true or bizarre kidney shapes. The nuclear character of the mature monocyte is illustrated in figures 2 *M* and 3.

(Contrast the mature monocytes with the myeloid elements shown in figure 4.)

*Wright-Stained Blood Films.*—Study of the Wright-stained blood films revealed essentially the same nuclear characters, although these lacked the sharp contrast of outlines achieved by the silver impregnation method. The majority of the Wright-stained cells showed the appearance of typical monocytes, with indented or folded nuclei and faintly basophilic cytoplasm stippled with fine, azure granules and rods. The

cytoplasm stained a dull grayish blue and presented faintly stained azure granules and filaments. Rarely a cell with the hemohistioblastic sponge-like nucleus was seen, with eosinophilic granules. Practically all of the cells of the monocyte series showed azure granules or filaments in the cytoplasm.

#### ASSOCIATION OF THE MONOCYTE AND HEMOHISTIOBLAST

The association of the monocyte and hemohistioblast in the circulating blood in various pathologic conditions has been observed by a number of investigators. Ferrata and Negreiros-Rinaldi<sup>18</sup> noted the association of the hemohistioblast and the monocyte in malaria. Some of the cells



Fig. 4.—A group of cells in a case of myelogenous leukemia including a myeloblast, two myelocytes, a young eosinophilic polymorphonuclear leukocyte and a neutrophilic polymorphonuclear leukocyte. Note that the chromatin is coarser and denser in the myelocytes than in the monocytes illustrated in the other figures. Photomicrograph;  $\times 1,750$ .

described by these authors were, however, undoubtedly macrophages. De Souza Aranha<sup>11</sup> observed, in the blood of a person with malaria and syphilis, true hemohistioblasts of a type identical with that of the hemohistioblasts in leukemia accompanying monocytosis. Richter<sup>12</sup> observed what he characterized as monocytoid hemohistioblasts in cases of quartan malaria and staphylococcus septicemia, and considered that all stages from the hemohistioblast to the monocyte were present. Many of the cells described by him were, however, phagocytic, and it is probable that some of the cells he observed were macrophages (histiocytes).

18. Ferrata, A., and Negreiros-Rinaldi: *Haematologica* 1:243, 1920.

Although Dameshek<sup>19</sup> failed to draw a line of distinction between the hemohistioblast of Ferrata and the macrophage, he undoubtedly observed the association of the hemohistioblast with the monocyte in monocytic leukemia. He noted specifically the presence of the hemohistioblast with the "spongy" nucleus in two cases of monocytic leukemia.

Reitano,<sup>7</sup> as previously noted, reported the presence of the hemohistioblast in monocytic leukemia and considered the monocyte to be derived from this cell, without, however, describing intermediate phases of differentiation. This author described the hemohistioblast in myelogenous leukemia as appearing in the different types, i. e., without granules, with azure granules, and with neutrophilic or eosinophilic granules.

Merklen and Wolf,<sup>20</sup> in a rather extensive study of the subject of monocytic leukemia, reported a case in which there were 20,500 white blood cells per cubic millimeter with 71 per cent monocytes or monoblasts, 14 per cent cells of the Ferrata type (hemohistioblasts), 14 per cent lymphocytes and 1 per cent myelocytes. These authors expressed the opinion that the Ferrata cells probably represented fragile monoblasts. Forkner<sup>21</sup> showed that the so-called monoblast is closely related to the undifferentiated mesenchymal cell. It would seem more probable that the Ferrata cells seen by Merklen and Wolf represented an earlier stage than the monoblast, or true mesenchymal cells. Ferrata<sup>5</sup> called attention to the similarity in nuclear architecture of the hemohistioblast and the mesenchymal cell. This frequently recorded association of hemohistioblast and monocyte cannot be considered mere chance, and the logical conclusion would be that they are genetically related.

#### COMMENT

The origin of the monocyte has been assigned variously to the myeloblast, the common or the specific endothelium, the lymphocyte and an undifferentiated mesenchymal cell.

Naegeli<sup>14</sup> considered the cell a member of the myeloid series arising from the myeloblast. McJunkin<sup>22</sup> and others expressed the belief that the monocyte is derived from common endothelium. Aschoff<sup>23</sup> and Kiyono<sup>24</sup> placed the monocyte in close relation with the specific endothelium (reticulo-endothelium) and its derivatives, the histiocytes

19. Dameshek, W.: *Arch. Int. Med.* **46**:718, 1930.

20. Merklen, P., and Wolf, M.: *Rev. de méd.* **45**:154, 1928; *Arch. d. mal. du cœur* **21**:129, 1928.

21. Forkner, C.: *J. Exper. Med.* **52**:3, 1930.

22. McJunkin, F.: *Am. J. Anat.* **25**:27, 1919.

23. Aschoff, L.: *Verhandl. d. deutsch. path. Gesellsch.* **16**:107, 1913.

24. Kiyono, K.: *Die Vital Karminspeicherung*, Jena, Gustav Fischer, 1914.

or macrophages. Bloom<sup>25</sup> considered the monocyte to arise through intravascular transformation of the small lymphocyte. Maximow,<sup>26</sup> too, believed an origin from the lymphocyte to be most probable, but admitted the possibility of an origin from the histiocyte or from an undifferentiated mesenchymal cell.

Cunningham, Sabin and Doan<sup>27</sup> described an origin of the monocyte from a primitive blood cell that itself is closely related to undifferentiated mesenchymal elements in the hematopoietic organs. Forkner<sup>21</sup> made a careful study of the origin of monocytes in the peripheral lymph nodes of the normal rabbit, and concluded that they arose from primitive, undifferentiated mesenchymal cells, passing through a stage which he calls a premonocyte, into the monocyte. He found no evidence that they arose under normal conditions from the macrophage (histiocyte) or from the reticulo-endothelium. He, like Simpson,<sup>28</sup> did, however, consider that the monocyte might be transformed into a macrophage. Forkner failed to find any evidence that the monocyte arose from the myeloblast, confirming the previous observations of Sabin and Doan.<sup>29</sup>

This study, tracing the development of the nucleus of the monocyte from a stem cell corresponding in nuclear architecture to the hemohistioblast, would appear to strengthen the position of the hemohistioblast as a true stem cell. Accepting the observation of Ferrata<sup>5</sup> that the hemohistioblast corresponds to the cell of the embryonic mesenchyme, this study consequently would favor the view held by Cunningham, Sabin and Doan<sup>27</sup> and Forkner<sup>21</sup> that the precursor of the monocyte is an undifferentiated mesenchymal cell. The observations recorded, of course, do not define the limits of the tissues from which cells of mesenchymal type may arise. They do not preclude the possibility of the reticulo-endothelium, under the stimulus of proliferation, being this source. Indeed, the observations of Merklen and Wolf, previously cited, would favor this view.

#### SUMMARY

By application of a silver impregnation method of staining to blood films in a case of monocytic leukemia, the monocyte has been traced to a stem cell corresponding to the hemohistioblast of Ferrata. A graded series of intermediate forms exists between the round or oval, lacelike nucleus characteristic of the hemohistioblast and the folded, skeinlike nucleus of the mature monocyte.

Miss Adelaide M. Hayes prepared the photomicrographs used in this article.

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25. Bloom, W.: *Folia haemat.* **37:1**, 1928.

26. Maximow, A.: *Arch. Path.* **4:4**, 1927.

27. Cunningham, R. S.; Sabin, F. R., and Doan, C. A.: *The Development of Leucocytes, Lymphocytes and Monocytes from a Specific Stem Cell in Adult Tissues*, Washington, D. C., Carnegie Institution, 1925, publication no. 361, p. 227.

28. Simpson, M.: *J. M. Research* **43:77**, 1922.

29. Sabin, F. R., and Doan, C. A.: *Proc. Soc. Exper. Biol. & Med.* **25:121**, 1927.

# EXPERIMENTAL ABSCESES IN THE RABBIT

## CYTOLOGY AND DRAINAGE

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Experimental abscesses are easily produced in man, and they are frequently produced for therapeutic purposes by the subcutaneous administration of turpentine. In animals, however, such abscesses are less easily produced. Hohnfeldt<sup>1</sup> produced them by the injection of a culture of staphylococci in an emulsion of agar, and Kiyono<sup>2</sup> inserted small pieces of sponges soaked either in a culture of staphylococci or in turpentine intramuscularly to produce pyogenic membranes. The literature on the cytology of these inflammatory reactions is enormous, and I shall not attempt to review it. The reader is referred to the excellent reports by Sabin<sup>3</sup> and by Maximow.<sup>4</sup>

Although the cytologic reaction in a given field of inflammation is generally well known, fewer data are available concerning absorption from such lesions. It is generally known that the absorption of a crystalloid or true solution takes place by way of the blood stream, but that the absorption of colloid solutions or suspensions of particulate materials is a function of the lymphatic radicles. It is probably true that absorption from an inflammatory region differs considerably from the normal, in that there are periods when complete occlusion of either lymphatic or venous radicles occurs. Rokitansky<sup>5</sup> maintained that absorption and inflammation are incompatible, and that inflammation must subside before any absorption from an inflammatory region can take place.

This study was undertaken to determine the extent and rate of absorption of both crystalloid and colloid solutions from experimental abscesses at varying intervals after their development.

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Work done in the Division of Experimental Surgery and Pathology.

1. Hohnfeldt, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **3**:343, 1888.

2. Kiyono, K.: *Die vitale Karminspeicherung*, Jena, Gustav Fischer, 1914, p. 94.

3. Sabin, Florence R.: *Bull. Johns Hopkins Hosp.* **34**:277, 1923.

4. Maximow, A. A.: *Arch. Path.* **4**:557, 1927.

5. Rokitansky, Carl: *A Manual of Pathological Anatomy*, London, Sydenham Society, 1850, vol. 3, p. 23.



## METHODS

Turpentine and croton oil, either pure or in various dilutions with refined cotton seed oil, when injected subcutaneously into the lumbar region, produced too diffuse a type of inflammation and were discarded as unsatisfactory. Various dilutions of aleuronat, however, were tried, and experience showed that a fresh 8 per cent solution was most successful in producing definitely localized inflammatory reactions. Intracutaneous injections induced necrosis of the superficial layers of the skin, subsequent evacuation and rapid healing. Intramuscular injections induced a too diffuse reaction, and there was much less cytologic response from muscle than from loose areolar tissue. Subcutaneous injections proved most satisfactory, and a site in the region of the loin, less disturbed by muscular activity, was selected for study. A preparation of graphite, known as "hydrokollog 300," and a 2 per cent solution of trypan blue were used as vital dyes during these observations; epinephrine hydrochloride and phenolsulphonphthalein were also used to test the absorption from the lesions.

## OBSERVATIONS

Cellular reactions induced subcutaneously in the loin in rabbits by aleuronat were such as characterize any field of inflammation. During the first two days neutrophilic leukocytes predominated, and there was partial necrosis along the inner part of the pyogenic wall. After the second day, however, there was marked decrease in the number of these granulocytes with an accompanying increase in lymphocytes, monocytes and macrophages. Ordinarily about the seventh day, but occasionally before, large numbers of eosinophilic leukocytes were present. Likewise, during the latter periods, fibroblasts were abundant.

When a preparation of graphite or a solution of trypan blue was injected into the posterior region of the loin in normal rabbits, the material was readily absorbed and was very easily traced through lymph vessels to regional lymph nodes. The iliac nodes were ordinarily first discolored, but the inguinal nodes were also occasionally involved. From the iliac lymph nodes vessels were easily traced to the lower lumbar nodes and thence to the cisterna chyli and the thoracic duct. If, however, either graphite or trypan blue was injected into an abscess a day old, pigment was never identified in any of the regional lymph nodes until two weeks had elapsed.

A series of abscesses was studied at successive intervals after the injection of graphite into them, in order to determine the dispersion through the lesion and the extent of the absorption. At five and seven days after the injection of aleuronat there was no absorption, and microscopic observations showed complete thrombosis of the blood vessels and lymph vessels (fig. 1). At nine days considerable cellular activity was displayed as evidenced by the dispersion of graphite, and gradually thrombi were removed; by the sixteenth day both blood and lymph vessels were again patent (fig. 2). Occasionally macrophages containing pigment were found in small vessels, but it was clear that most of the injected pigment was removed by way of the lymph stream.



Fig. 1.—Abscess of seven days' duration. There is graphite against the inner wall, with no dispersion; the vessels are thrombosed;  $\times 65$ .

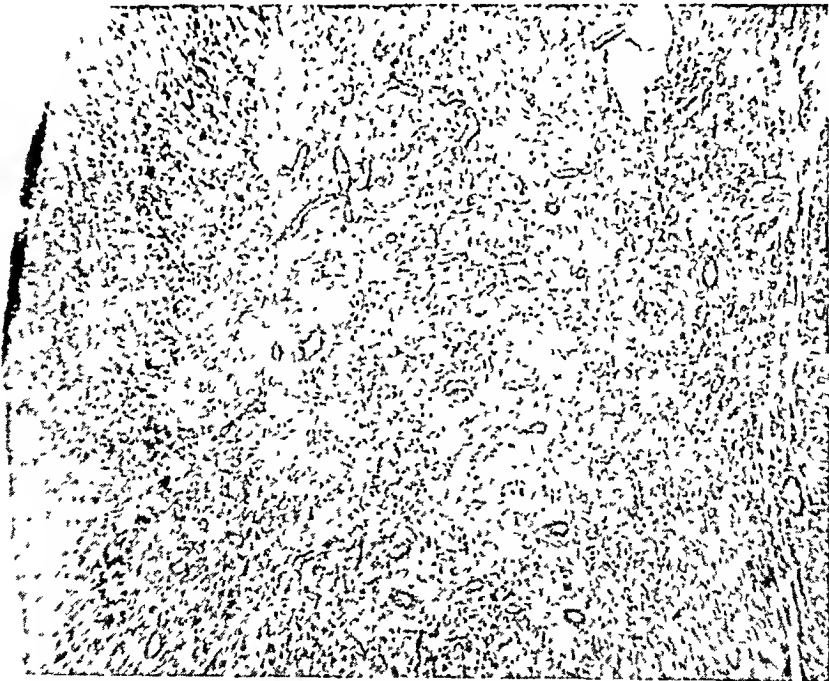


Fig 2.—Abscess of sixteen days' duration. Graphite is scattered through the wall, and the vessels are patent;  $\times 32$

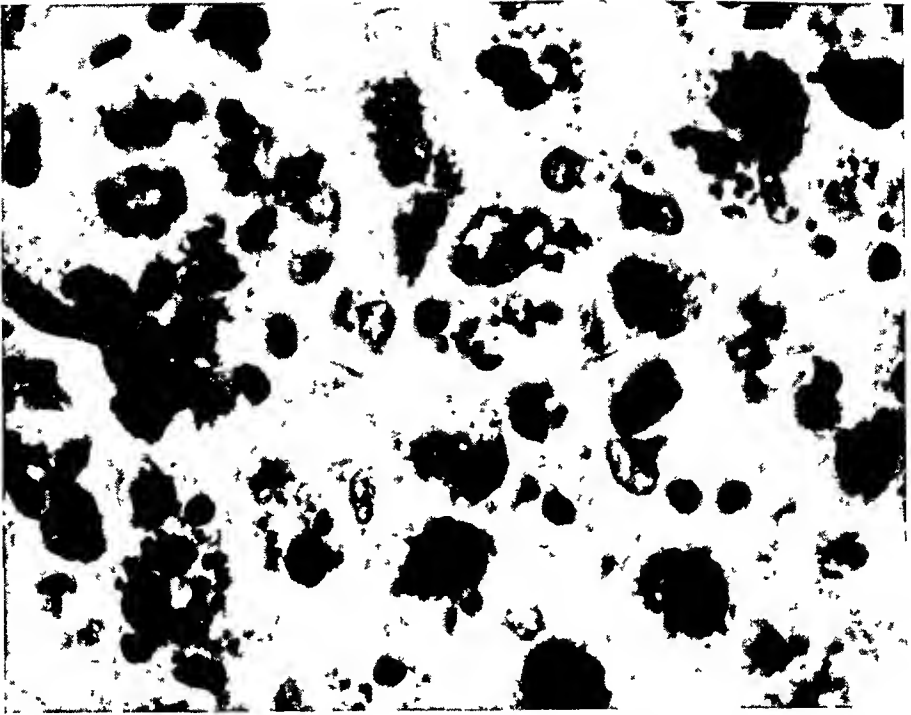


Fig. 3.—Macrophages with trypan blue in an abscess 28 days old. The granules are in rosette formation;  $\times 700$ .

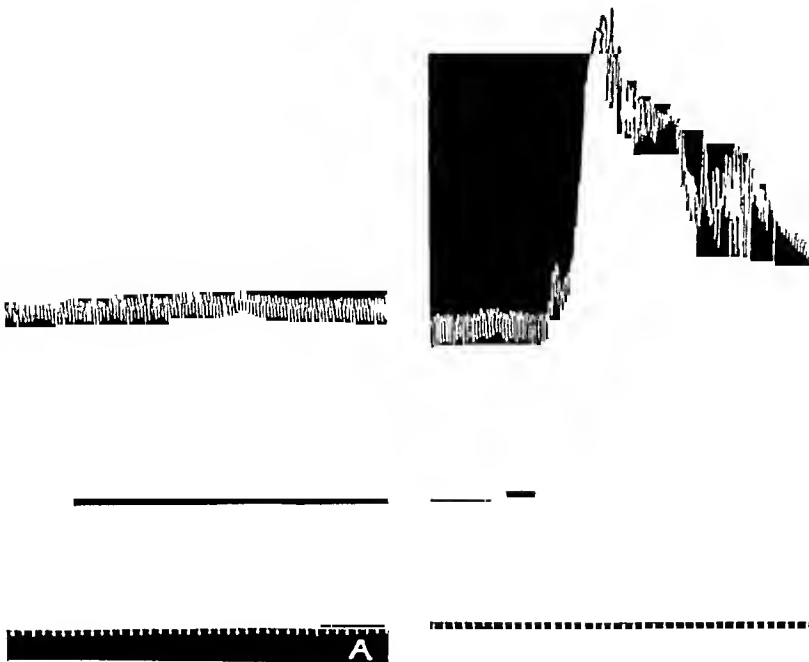


Fig. 4.—The response in blood pressure to an injection of epinephrine hydrochloride. *A* represents the response in a rabbit in which 1 cc. of a 1:1,000 dilution of epinephrine was injected into a normal subcutaneous area; *B*, that of a rabbit in which the same dose was injected into the outer wall of an abscess. Epinephrine hydrochloride was absorbed quickly when injected into the hyperemic wall of the abscess, which was 28 days old.

A comparable result was attained in a series of abscesses, from 1 to 34 days old, into which 2 cc. of a 2 per cent solution of trypan blue was injected a few days before necropsy (fig. 3). It was clear from both gross and microscopic studies that absorption, as revealed by the presence of the dye in lymph vessels and lymph nodes, did not occur until the fourteenth day. Thereafter, and until the concluding observations were made on an abscess 35 days old, pigment-laden macrophages identical with those seen in the abscess itself were recovered from the related lymph nodes.

Besides the vital dyes, various drugs were used to test absorption from these experimental abscesses. Luckhardt and Koppányi<sup>6</sup> showed

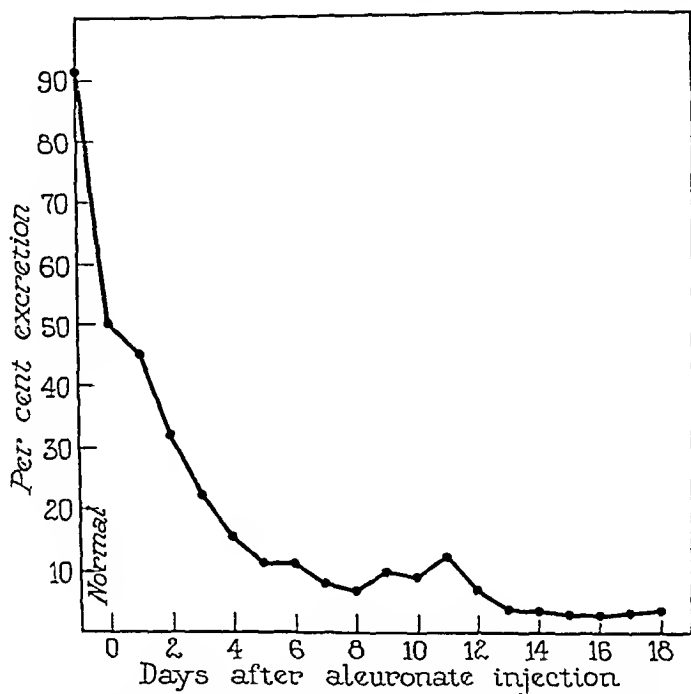


Fig. 5.—The average daily absorption and excretion of phenolsulphonphthalein from abscesses of six rabbits.

that a rise in blood pressure followed the subcutaneous injection of epinephrine hydrochloride into dogs only when the region was massaged. Accordingly, with animals anesthetized with sodium iso-amyl-ethyl barbiturate (sodium amytal) determinations of blood pressure were made directly from the carotid artery in animals that had received small dilutions of epinephrine into subcutaneous spaces as well as into abscessed areas. It was clear that absorption of the drug from abscesses a few days old could not be detected by any changes in blood pressure. On the other hand, a marked rise in blood pressure was obtained follow-

6. Luckhardt, A. B., and Koppányi, Theodore: *Am. J. Physiol.* **81**:436, 1927.

ing the injection of a comparable solution of epinephrine into the cavity of an abscess 4 weeks old (fig. 4).

Colorimetric tests of samples of urine, as a means of determining the extent of absorption of phenolsulphonphthalein from subcutaneous areas of normal rabbits and from abscesses in varying stages of development, gave essentially the same results. When the dye was injected daily into experimental abscesses from the time of their inception up to thirty days, and two hour samples of urine compared with a standard solution, I observed a persistent decrease in the amount of absorption until about the sixth or eighth day. Forty-five per cent of the dye introduced into an abscess 1 day old was recovered from the urine; but this percentage dropped progressively until the sixth or eighth day and remained as low as from 3 to 5 per cent until about the twentieth day (fig. 5). In normal rabbits, serving as controls, daily absorption and excretion of the dye from these same regions occurred at rates varying from 70 to 90 per cent.

#### COMMENT

These observations fully substantiate the conclusions hitherto stated in the literature, that absorption from subcutaneous regions takes place either through the lymph channels or through the blood capillaries, and that the route taken by an absorbed material is to a large extent determined by the physical characteristics of the substances absorbed. In other words, the absorption of particulate, granular material or of colloidal suspensions is to a large extent a function of the lymphatic system, whereas true solutions, or even finely divided colloidal solutions, are absorbed into the blood vascular system, and eliminated from the body by the components of the excretory system.

#### SUMMARY

The difference in absorption from an abscess as compared with a normal subcutaneous area is one of degree rather than of mechanism. The blockage established for particulate materials is regional and complete between the first and ninth days. The blockage for soluble materials is limited to the inner wall of the abscess and is not established until after from four to six days, after which the excretion remains low. An experimental abscess is most efficiently walled off between the sixth and twelfth days.

# PHARYNGO-ESOPHAGEAL DIVERTICULUM

WITH SUBDIVERTICULAR ESOPHAGEAL STENOSIS, FOREIGN BODY  
IMPACTION AND SUDDEN DEATH: REPORT OF A CASE

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Much has been written in recent years about esophageal diverticulum, especially about the "boundary diverticulum," or pharyngo-esophageal diverticulum. The following report of a case is submitted because of its bearing on the etiology and termination of this condition.

## REPORT OF CASE

A well developed white man, aged 61, came to coroner's autopsy. He was unmarried and lived alone. It was believed by the coroner that he had not eaten previously for three days because of lack of funds. He entered a cheap restaurant, and while eating a twenty-five cent dinner suddenly arose and without a sound walked toward the door, where he fell to the floor and was dead when help arrived. None of those present noticed him choking or any peculiarity in his manner of eating. His relatives stated that he had been in excellent health.

*Autopsy.*—The coelomic organs were examined first. There was no change in the heart or great vessels with the exception of acute dilatation of the right chambers of the heart. The lungs were large and rather boggy posteriorly, but free from consolidations. When they were sectioned, the surfaces were deep red and moist, and abundant dark red fluid dripped away. The stomach was moderately distended with poorly masticated food. There were a few capillary hemorrhages dotting the mucosa, but nothing else of importance was seen.

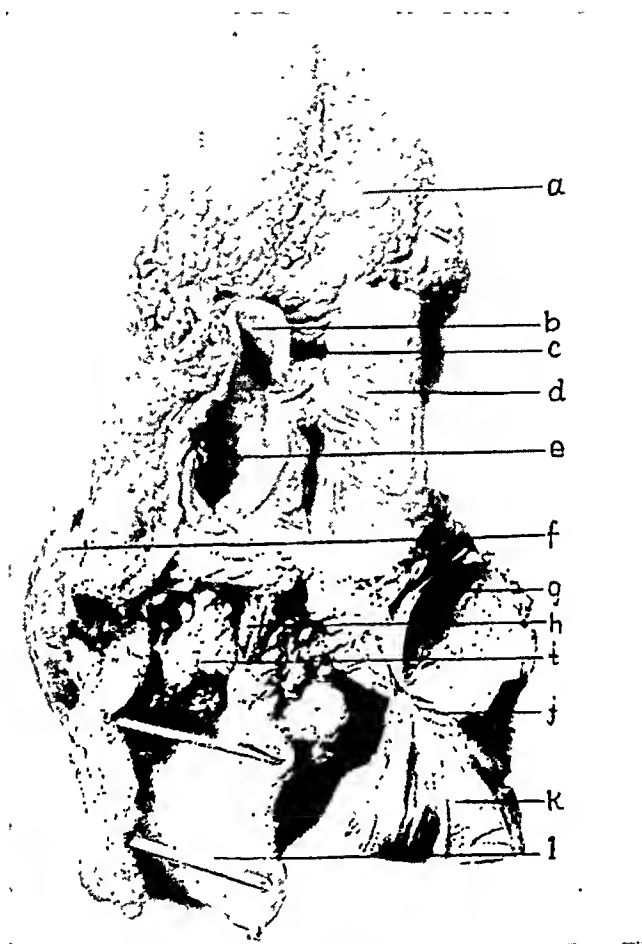
When the organs of the neck were removed en masse, there was seen a bulging of the posterior wall of the pharynx, with its lowest portion 1 cm. below the lower border of the cricoid cartilage, or 8 cm. below the tip of the epiglottis. The structure stood 1.5 cm. above the level of the adjacent esophagus and measured 4.5 cm. in length and 3.4 cm. in breadth (figure). As the fascia was carefully dissected away, the longitudinal muscle layer of the esophagus was found to fan out over the lower border of the sac, leaving a few scattered muscle fibers over the main mass, while on each side laterally was a heavy bundle of skeletal muscle forming an erect "Y." The upper border of the sac was on the same level as the superior border of the cricoid. Here the horizontal muscle fibers of the inferior pharyngeal constrictor crossed over the superior border of the sac. The long axis of the sac was directed posteriorly and inferiorly, with only the lower 1 cm. overhanging the posterior esophageal wall. When the sac was laid open (figure), it was found packed with shreds of poorly masticated meat. At the inferior border of the sac was a constriction of the mouth of the esophagus with a diameter of 0.4 cm. and a circumference of 1.3 cm. The opening from the pharynx into the diverticulum

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measured 4 cm. in circumference. Grossly there appeared to be only slight fibrous thickening in the submucosa, with a moderately increased circular layer of muscle outside this in the esophageal wall. The stricture was 0.9 cm. in length; the esophagus rapidly widened below to a circumference of 4.5 cm. within a distance of 2 cm. below the stricture. The wall of the sac varied from 1 to 2 mm. in thickness. The lining epithelium was gray and slightly roughened. The remainder of the wall was composed of slightly thickened fibrous tissue with little, if any, muscle. The posterior pharynx and pyriform recesses were likewise filled with



Photograph of the organs of the neck illustrating the essential points of interest of the esophageal diverticulum: (a) tongue, (b) epiglottis, (c) epiglottic vallecula, (d) pharyngeal wall (dissected laterally), (e) laryngeal aditus, (f) left thyroid lobe, (g) anterior view of diverticulum (dissected laterally), (h) cricoid cartilage, (i) mass of meat in larynx, (j) subdiverticular stricture, (k) esophagus and (l) trachea.

poorly masticated meat extending through the glottis and into the larynx. Wedged between the vocal cords, entirely filling the larynx, was a single tough mass of incompletely chewed meat and fascia measuring 4 by 3 by 1.5 cm. Beneath this in the trachea and main bronchi were other smaller pieces and shreds of tough meat, together with an abundance of mucus. The lining mucosae of the trachea, larynx and pharynx were pale, pinkish gray and smooth, and showed no swelling.

Microscopic sections cut longitudinally through the narrowed point revealed the intact covering of stratified squamous epithelium with no horny layer. There was no thickening of the lamina propria; a moderate increase in the normal content of lymphocytes and scattered plasma cells were observed here. The muscularis mucosae was composed of intermittent rather heavy bands of smooth muscle disposed longitudinally, and was absent in intervening areas. The submucosa was composed of loose fibrous tissue, free from cellular infiltration and not appreciably thickened. There were no glands present. The lamina muscularis measured 1.5 to 2.5 mm. in thickness. The inner circular layer blended imperceptibly with the outer thin longitudinal fibers through intervening oblique strands. The tunica externa was made up of loose fibro-areolar connective tissue mixed with fat. There was no cellular infiltration here.

Horizontal sections taken from the lower end of the wall of the diverticulum revealed the mucosa to be slightly redundant and folded on itself. The loose fibro-areolar tissue of the mucosa and submucosa merged without demarcation owing to the absence of muscularis mucosae or elastic membrane. An abundant lymphocytic infiltration was found below the epithelium at one point. Deep beneath this was a solitary lymph follicle with hyperplastic germinal center. A single clump of mucous glands infiltrated heavily by small lymphocytes was seen. Leading away from this was a large duct lined by a double to triple layer of cuboidal epithelium containing in the lumen a small quantity of amorphous, pale-staining material with a small quantity of yellow granular pigment. Closely surrounding the duct were numerous plasma cells. There was a thin, incomplete layer of irregularly disposed voluntary muscle with predominant circular arrangement. In some places, this failed completely and was supplanted by loose fibrous tissue, with an occasional muscle fiber or bundle circularly placed. Only a few isolated longitudinal muscle fibers were present.

Sections cut horizontally from the midportion of the diverticulum showed a transition from normal, noncornifying, stratified squamous epithelium on one end, through a patch of partially necrotic epithelium with heavy neutrophilic infiltration to a large area of complete epithelial necrosis covered with polymorphonuclear leukocytes entangled in fibrin. Numerous neutrophils and eosinophils were found here and in the connective tissue just beneath. In the underlying loose connective tissue, the capillaries were dilated, and the tissue meshes were filled with plasma cells, round cells and neutrophils in about equal numbers. Several hyperplastic solitary lymph follicles lay in this region. Again, outside this, was a rather thick layer of loose fibrous connective tissue containing numerous arterioles, the walls of which were thickened by almost acellular hyaline connective tissue. Only scattered plasma cells and histiocytes were encountered here. There was a single group of skeletal muscle fibers, which were placed in longitudinal manner.

#### COMMENT

The etiology of pharyngo-esophageal diverticulum has long been disputed, some authors<sup>1</sup> holding that the outpocketing is congenital or due to congenital defect, while others<sup>2</sup> have maintained that it is due to

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1. Mosher, H. P.: *Laryngoscope* **34**:854, 1924. Oehlecker, F.: *Arch. f. klin. Chir.* **134**:699, 1925.

2. Kraas, E.: *Klin. Wchnschr.* **9**:1457, 1930. Lotheissen, George: *Ergebn. d. Chir. u. Orthop.* **23**:110, 1930.



mechanical means, by evagination of the mucosa and submucosa through a weak point in the muscular wall in the presence of increased intra-esophageal pressure. This weak point is the triangle described by Laimer (cited by Lotheissen<sup>2</sup>) on the posterior pharyngeal wall at the union of the esophagus and pharynx where the longitudinal esophageal muscle fibers diverge to unite with the lower fibers of the inferior pharyngeal constrictor.

Boundary diverticulum has been reported in conjunction with esophageal stenosis of various kinds. Several cases associated with compression of the esophagus by retrosternal goiter<sup>3</sup> have been recorded. Others<sup>4</sup> have described instances of pharyngo-esophageal diverticula associated with cardiospasm, and in addition Zohlen cited a personal communication from Vinson of a fourth similar case. Mosher<sup>1</sup> described one case of congenital malformation of the pharynx associated with diverticulum, and ascribed others to developmental errors of this kind because of the frequency of malformations of the pharynx in the cadaver. Chevalier Jackson,<sup>5</sup> from his vast experience with internal examination of the esophagus during life, considered the failure of the cricopharyngeus muscle to relax its normal tone during deglutition as the most important exciting factor in the formation of boundary diverticula. Sturgeon<sup>6</sup> concurred in this view. Keiper<sup>7</sup> described the anatomic changes in a case of cicatricial stenosis of the esophagus associated with cervical pulsion diverticulum, and ascribed the sacculatation to this origin in most instances. However, no other similar case proved by autopsy has been found in a search of the literature, although the clinical findings in several instances<sup>8</sup> have suggested this etiology.

All these citations suggest that increased intra-esophageal or intra-pharyngeal pressure in conjunction with obstruction of the upper alimentary canal from the various causes may be important in the formation of this type of diverticulum. However, it is well known that marked and prolonged obstruction of the esophagus may exist with no sign of diverticularization. Vinson<sup>9</sup> listed 186 cases of benign cicatricial stenosis of the esophagus seen at the Mayo Clinic, but made

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3. Bouvier, E.: *Arch. f. klin. Chir.* **134**:802, 1925. Lerche, W.: *S. Clin. North America* **3**:1227, 1923. Haberer: *Arch. f. klin. Chir.* **122**:789, 1923; abstr., *J. A. M. A.* **80**:1109, 1923.

4. Fitzgibbon, J. H.: *J. A. M. A.* **86**:1614, 1926. Zohlen, J. P.: *Wisconsin M. J.* **28**:526, 1929. Abell, I.: *S. Clin. North America* **10**:901, 1930.

5. Jackson, Chevalier, and Shallow, T. A.: *Ann. Surg.* **83**:1, 1926.

6. Sturgeon, C. T.: *J. A. M. A.* **92**:379, 1929.

7. Keiper, G. F.: *Laryngoscope* **22**:1127, 1912.

8. Gaub, O. C., and Jackson, Chevalier: *Surg., Gynec. & Obst.* **21**:52, 1915. Kuster: *Arch. f. klin. Chir.* **83**:613, 1907. Sturgeon (footnote 6).

9. Vinson, P. P.: *Ann. Otol., Rhin. & Laryng.* **36**:40, 1927.

no mention of associated diverticula in any. Downie<sup>10</sup> also failed to note this complication in a similar discussion of 100 consecutive obstructions of the gullet.

It then seems evident that some factor other than obstruction, possibly weakening of the wall or congenital malformation, is necessary in diverticularization. The case now reported is an example of obstruction of the esophagus and early diverticulum occurring together. In deciding the etiology of the stenosis in this case, the absence microscopically of scar tissue or cellular infiltration in the narrowed area is against postinflammatory stricture. There is no evidence of malignant growth. The relatives, when closely questioned, stated that the dead man had never complained of difficulty in swallowing but that three years before death he was observed to wash down each bolus with liquid; they told of a choking spasm that he had suffered while eating six months prior to death. Since no abnormality had been noticed before this, a congenital stenosis is unlikely. It may be fairly assumed, then, that one is dealing with a spastic closure due to increased muscle tonus. Even here one is on uncertain ground, as Mosher<sup>1</sup> stated that in an unfixed specimen from a cadaver, stenosis of the esophageal orifice is produced by backward pressure on the posterior pharyngeal wall. The sudden death without evidence externally of choking resulting from complete closure of the glottis in the absence of demonstrable heart disease is of medicolegal interest. The apparent rapidity with which pulmonary congestion occurred is striking. The recent acute inflammatory process in the wall of the diverticulum was probably due to decomposition of residual food or secretion in the sac.

#### SUMMARY

An instance of pharyngo-esophageal diverticulum with subdiverticular esophageal stenosis, foreign body impaction and sudden death is recorded, apparently due to muscle spasm in the esophagus with resulting increased pulsion in the posterior pharynx during deglutition.

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10. Downie, W.: *Glasgow M. J.* 87:336, 1912.

# EXPERIMENTAL THROMBO-ANGIITIS OBLITERANS

## BACTERIOLOGIC AND PATHOLOGIC STUDIES

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In a consideration of the causes of thrombo-angiitis obliterans, infection seems the most logical cause, in which event the infection will be of a distinctly low grade, as is shown by the usual picture of pathologic change.<sup>1</sup> In a study of the arteries and veins in this disease, one is impressed with the inflammatory nature of the lesions. Buerger<sup>2</sup> stated his belief that the disease is due to a specific infectious or toxic agent. He has succeeded in reproducing the lesion in human beings by inoculating material from acutely inflamed superficial veins of patients with thrombo-angiitis obliterans adjacent to superficial normal veins of patients who previously had had thrombo-angiitis obliterans; the lesions were not produced when he injected the material into a tied loop of a superficial vein. However, a specific agent or organism was not isolated from these lesions. He obtained negative results in animals. Rabinowitz<sup>3</sup> claimed to have found a gram-negative bacillus in the blood of patients with thrombo-angiitis obliterans and to have produced vascular lesions in rabbits. Jablons<sup>4</sup> was unable to confirm this work. The work which we report was started in November, 1927, and was continued for a period of two and a half years. It seems desirable at this time to report the relatively large number of data which has accumulated during this period.

## MATERIAL AND METHODS

The material used in this investigation was obtained from fifty-six patients with thrombo-angiitis obliterans and ten patients with arteriosclerotic disease of the lower extremities. The youngest patient with thrombo-angiitis obliterans was aged 23 years, and the oldest, 73 years. One patient was a woman, aged 60 years; the

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From the Division of Medicine of the Mayo Clinic.

1. Brown, G. E.; Allen, E. V., and Mahorner, H. R.: *Thrombo-Angiitis Obliterans*, Philadelphia, W. B. Saunders Company, 1928.

2. Buerger, Leo: *Surg., Gynec. & Obst.* **19**:582, 1914; *Arch. Path.* **7**:381, 1929.

3. Rabinowitz, H. M.: *Surg., Gynec. & Obst.* **37**:353, 1923.

4. Jablons, Benjamin: *Internat. Clin.* **3**:193, 1925.

others were men. Seventeen of the patients with thrombo-angiitis obliterans had had a leg amputated, and one patient had had a finger amputated. Gangrene was present in each extremity that was amputated. Nineteen acutely inflamed superficial veins were obtained at biopsy from the extremities of a corresponding number of patients who had thrombo-angiitis obliterans. All of the patients with arteriosclerotic disease had had an extremity amputated. In both groups, large segments of arteries and veins were dissected from the amputated extremity at the time of operation, placed in a sterile test tube and immediately taken to the laboratory, where cultures were made. A large number of sections was preserved for microscopic study. After the tissues had been washed several times with a solution of sterile physiologic solution of sodium chloride, numerous segments were embedded in tubes of soft dextrose brain agar, which had been previously melted and cooled to 40 C. Other portions were emulsified in a mortar under aseptic precautions and inoculated into tall tubes of dextrose brain broth and dextrose brain agar, the mediums of choice used by Rosenow<sup>5</sup> in studies of elective localization. These were then sealed with sterile petrolatum, to insure anaerobic conditions, and incubated at 37.5 C. for from twenty-four hours to five months. Subcultures were not made until the cultures showed evidence of growth. For a control series cultures were made of normal arteries and veins obtained from twenty-four other patients at surgical operations. These tissues were cultured in the same manner as those obtained from patients having thrombo-angiitis obliterans and arteriosclerosis.

#### RESULTS OF CULTURE

Cultures obtained from segments of veins and arteries from seventeen amputated extremities of patients who had thrombo-angiitis obliterans gave the following results: Gram-positive, pleomorphic streptococci were obtained in four instances (fig. 1 *A*), green-producing streptococci in two instances, staphylococci in three, and gram-positive bacilli in four.

Cultures were made from segments of nineteen acutely inflamed superficial veins obtained at biopsy from a corresponding number of patients with thrombo-angiitis obliterans; from three to seven cultures were made from each specimen (table 1). Gram-positive, pleomorphic streptococci were isolated from six specimens, staphylococci from seven specimens, and gram-positive bacilli from three specimens.

The cultures from an acutely inflamed vein obtained at biopsy from a man, aged 52 years (case 3, table 1), showed gram-positive, pleomorphic streptococci on the eighth day of incubation. This patient returned to the clinic three months later, and the left leg was amputated below the knee. Organisms isolated from the deep arteries and veins of the amputated extremity on the eighteenth day after incubation were morphologically the same as those previously obtained from the specimen used for biopsy.

Approximately from five to thirty-five hours after the acutely inflamed veins were first observed, they were removed surgically under

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5. Rosenow, E. C.: Personal communication.

aseptic conditions. In each instance, the superficial vein and surrounding skin showed definite evidence of an inflammatory process. The skin was red, swollen and tender to palpation, and a small segment of the vessel was obviously thrombosed, as the vessel both proximal and distal to the inflamed area could be collapsed with gentle pressure, whereas the area of thrombosis appeared as a solid cord. Occasionally, the area of thrombosis could be extruded from the cut end of the vein by gentle pressure at the time of the taking of the specimen for biopsy, but in most instances the lesion was firmly adherent to the wall of the vessel.

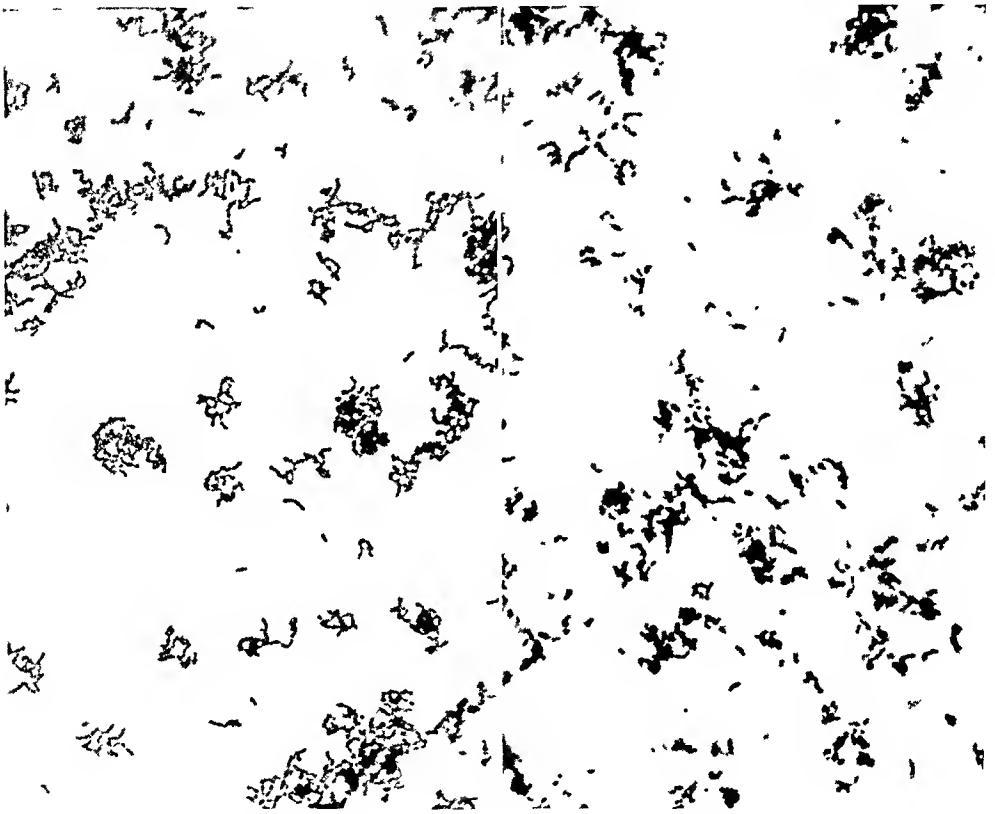


Fig. 1.—*A*, pleomorphic streptococci isolated from arteries and veins obtained at the site of amputation of a leg of a man, aged 52 years, who had thrombo-angitis obliterans (case 3, table 1);  $\times 1,000$ . *B*, pleomorphic streptococci isolated from arteries and veins obtained from an amputated leg of a man, aged 69 years (case 3, table 2), who had arteriosclerotic disease of the lower extremities;  $\times 1,000$ . Gangrene was not present in the leg.

The seventh day was the minimal time and the twenty-sixth day was the maximal time at which the pleomorphic streptococcus was isolated from patients with thrombo-angiitis obliterans. The green-producing streptococcus was isolated from the vessels of the amputated leg at the end of twenty-four hours and from the vessels of the amputated finger on the eleventh day. When first isolated, the pleomorphic streptococcus appeared as a slender, gram-positive, nonmotile

bacillus, occurring singly or in pairs, closely resembling diphtheroid bacilli. In subcultures in dextrose brain broth or dextrose brain agar, its morphologic character changed so that it appeared in short, gram-positive, coccoid chains. The average chain contained from three to five organisms. These organisms were always isolated under anaerobic conditions. In subcultures they grew only under anaerobic conditions, except in three instances in which after repeated cultures they grew

TABLE 1.—*Cultures Obtained from Arteries and Veins of Patients with Thrombo-Angitis Obliterans*

Patient	Age	Sex	Source of Tissue Cultured		Period of Incubation, Days	Results
			Biopsy	Amputation		
1	35	M	Vein	.....	10	Gram-positive pleomorphic streptococcus
2	23	M	Vein	.....	9	Gram-positive pleomorphic streptococcus
3	52	M	Vein	.....	8	Gram-positive pleomorphic streptococcus
				Gangrenous leg	18	Gram-positive pleomorphic streptococcus
4	25	M	Vein	.....	26	Gram-positive pleomorphic streptococcus
5	36	M	Vein	.....	16	Gram-positive pleomorphic streptococcus
6	31	M	.....	Gangrenous leg	11	Gram-positive pleomorphic streptococcus
7	33	M	.....	Gangrenous leg	9	Gram-positive pleomorphic streptococcus
8	36	M	.....	Gangrenous leg	17	Gram-positive pleomorphic streptococcus
9	39	M	Vein	.....	7	Gram-positive pleomorphic streptococcus
10	73	M	.....	Gangrenous leg	1	Green-producing streptococcus
11	25	M	.....	Gangrenous finger	11	Green-producing streptococcus
12	37	M	.....	Gangrenous leg	141	Four negative cultures
13	45	M	Vein	.....	132	Staphylococci in 1 of 5 cultures
				Gangrenous leg	82	Gram-positive cocci in 1 of 4 cultures
14	40	M	.....	Gangrenous leg	72	Five negative cultures
				Reamputation	57	Staphylococci in 1 of 5 cultures
15	42	M	.....	Gangrenous leg	68	Gram-positive bacilli in 1 of 5 cultures
16	49	M	.....	Gangrenous leg	68	Five negative cultures
17	36	M	.....	Gangrenous leg	53	Staphylococci and gram-positive bacilli in 1 of 6 cultures
18	34	M	Vein	.....	90	Six negative cultures
19	35	M	.....	Gangrenous leg	65	Six negative cultures
20	49	M	Vein	.....	120	Staphylococci in 1 of 3 cultures
21	55	M	.....	Gangrenous leg	57	Gram-positive bacilli in 1 of 5 cultures
22	57	M	Vein	.....	67	Four negative cultures
23	29	M	Vein	.....	43	Staphylococci in 3 of 5 cultures
24	60	F	Vein	.....	45	Gram-positive bacilli in 1 of 5 cultures
				Gangrenous leg	78	Ten negative cultures
				Gangrenous leg	104	Six negative cultures
25	38	M	.....	Gangrenous leg	69	Eight negative cultures
26	33	M	Vein	.....	55	Gram-positive bacilli in 3 of 6 cultures
27	52	M	.....	Gangrenous leg	102	Staphylococci in 3 of 7 cultures
28	49	M	Vein	.....	86	Five negative cultures
29	48	M	Vein	.....	81	Staphylococci in 2 of 6 cultures
30	31	M	Vein	.....	65	Staphylococci in 1 of 3 cultures
31	43	M	Vein	.....	87	Four negative cultures
32	34	M	Vein	.....	79	Staphylococci in 2 of 4 cultures
33	30	M	Vein	.....	104	Eight negative cultures
34	40	M	.....	Gangrenous leg		

slowly on the surface of horse blood agar plates. Except in these three instances, neither the primary cultures nor the subcultures were grown on blood agar plates under aerobic conditions. The colonies on blood agar plates appeared as colorless, pinpoint-sized, dry, translucent bodies, showing neither hemolysis nor formation of pigment. Subcultures in dextrose brain broth or dextrose brain agar also grew very slowly, the growth first appearing at from the third to the sixth day. The pleomorphic streptococcus was never found associated with other micro-organisms. The negative cultures were allowed to incubate for from sixty-five to one hundred and forty-one days at 37.5 C. The

green-producing streptococci grew readily in primary culture on blood agar plates and in dextrose brain broth. They also grew from the embedded vessels and emulsion in from twenty-four to forty-eight hours.

A similar study was made of arteries and veins from amputated extremities obtained from ten patients with arteriosclerotic disease of the lower extremities. The youngest patient was aged 49 years, and the oldest, 78 years. The vessels were obtained under aseptic conditions at the site of the amputation and were cultured in a manner similar to that previously described for the patients with thrombo-angiitis obliterans. Gram-positive, pleomorphic streptococci were isolated from the arteries and veins of five patients (fig. 1 *B*). Cultures from the arteries and veins of four did not contain organisms; one of ten cultures

TABLE 2.—*Cultures Obtained from Arteries and Veins of Ten Patients with Arteriosclerosis\**

Age	Sex	Cultures Incubated, Days	Results
68	M	24	Pleomorphic streptococcus
71	M	2	Pleomorphic streptococcus
69	M	21	Pleomorphic streptococcus
70	M	9	Pleomorphic streptococcus
71	M	9	Pleomorphic streptococcus
78	M	150	Four negative cultures
53	M	152	Two negative cultures
66	M	72	Five negative cultures
49	M	20	Gram-positive bacillus in 1 of 10 cultures
68	F	38	Six negative cultures

\* The arteries and veins were obtained at the time, and at the site, of amputation of the leg. Gangrene was present in one or more toes of all the extremities amputated with the exception of the third case.

showed gram-positive bacilli on the twentieth day of incubation. All of these cultures were incubated at 37.5 C. for from four to one hundred and fifty-two days. The earliest time at which the pleomorphic streptococci appeared was on the fourth day, and the latest time, the twenty-fourth day. A summary of the cultures of arteries and veins obtained from patients having arteriosclerosis is shown in table 2.

For a control study, cultures were made of normal arteries and veins obtained from twenty-four other patients in the course of various types of operations (table 3). Sixteen of the cultures did not yield growth; seven contained gram-positive cocci, and one, a gram-positive bacillus.

The pleomorphic streptococci obtained from nine patients with thrombo-angiitis obliterans were grown in 150 cc. of dextrose brain broth for from six to eighteen days at 37 C. From 0.1 to 0.5 cc. was inoculated into eleven different sugars, with results as follows: All fermented dextrose; four lactose; five, saccharose, salicin and maltose; two, mannite; six, raffinose; nine, galactose; eight, levulose, and eight,

inulin and rhamnose. Two produced acid in litmus milk, and two, acid and coagulation. The micro-organisms from all the patients grew readily on Drigalski-Conradi agar and litmus lactose agar. The colonies appeared very small and colorless and were embedded in the agar.

The pleomorphic streptococci obtained from arteries and veins of five patients with arteriosclerosis were inoculated into the same eleven sugars with results as follows: Five fermented dextrose; one, lactose; one, saccharose; none, salicin; one, maltose, mannite and raffinose; two, galactose; five, levulose; one, inulin, and one, rhamnose. One produced acid in litmus milk, and one, acid and coagulation. Growth appeared in

TABLE 3.—*Cultures Obtained from Arteries and Veins of Twenty-Four Normal Subjects*

Source of Tissue Cultured	Period of Incubation, Days	Results
Abdominal wall.....	160	Three negative cultures
Abdominal wall.....	160	Three negative cultures
Great omentum.....	160	Three negative cultures
Muscle from neck.....	154	Three negative cultures
Abdominal wall.....	154	Three negative cultures
Muscle from neck.....	9	Staphylococci in 1 of 3 cultures
Great omentum.....	154	Three negative cultures
Great omentum.....	154	Three negative cultures
Subcutaneous tissue from leg.....	8	Staphylococci in 1 of 3 cultures
Subcutaneous tissue from leg.....	5	Staphylococci in 1 of 3 cultures
Subcutaneous tissue from knee.....	134	Three negative cultures
Subcutaneous tissue from hip.....	122	Three negative cultures
Subcutaneous tissue from knee.....	91	Three negative cultures
Muscle from leg.....	1	Three cultures contained staphylococci
Muscle from hip.....	7	Gram-positive bacilli in 1 of 4 cultures
Muscle from leg.....	9	Staphylococci in 2 of 6 cultures
Muscle from leg.....	23	One negative culture
Muscle from leg.....	43	Two negative cultures
Abdominal wall.....	5	Staphylococci in 1 culture
Great omentum.....	60	One negative culture
Great omentum.....	60	One negative culture
Great omentum.....	5	Staphylococci in 1 culture
Great omentum.....	60	Two negative cultures
Great omentum.....	60	One negative culture

Drigalski-Conradi agar and litmus milk in all except one case. Growth was not observed on either potato or endo-agar plates.

*Comment.*—The pleomorphic streptococci that were obtained from the arteries and veins of patients with thrombo-angiitis obliterans and arteriosclerosis seemed to be morphologically identical, and the cultural characteristics were for the most part similar. It is the general belief that low grade infection is the etiologic basis for thrombo-angiitis obliterans, as is observed in the usual pathologic picture. On the other hand, most observers do not regard arteriosclerosis as of infectious origin. It is possible that in the cases included in this report under the heading "arteriosclerosis," a so-called mixed type of lesion may have been present, as was demonstrated in one of the five positive cultures of pleomorphic streptococci. The patient was a man, aged 71 years (case 2, table 2). Clinically and from microscopic study of the vessels, these cases must be classified as representing senile arteriosclerosis, but it is



possible that some of the patients may have had thrombo-angiitis obliterans in a mild form at an earlier date. This is borne out by a recent report on a study of thrombo-angiitis obliterans of aged patients.<sup>6</sup> One of the patients in this group, a man aged 73 years, had thrombo-angiitis obliterans, as was proved by study of the vessels of the amputated extremity and later at necropsy (case 10, table 1). Ordinarily from a clinical standpoint and from microscopic study of an occasional vessel, this patient would have been reported as having arteriosclerosis, primarily because of his age, but age itself does not exclude the presence of thrombo-angiitis obliterans. It is also true that large segments of vessels were used for culture, and no attempt was made to obtain serial sections of segments of similar size for microscopic study. This may be of definite significance, since the lesions of human beings with thrombo-angiitis obliterans are distinctly patchy in distribution. A small segment of a vessel may often be seen to be occluded, yet the vessel both proximal and distal to the lesion will appear to be normal. If any significance can be attached to the various organisms isolated in this study, it must be with reference to the pleomorphic streptococcus. The other organisms represent either contaminants or secondary invaders present because of gangrene.

#### RESULTS OF INJECTION IN ANIMALS

Experiments on animals seemed necessary in order to attempt to evaluate the etiologic significance of the pleomorphic streptococci that were obtained from the arteries and veins of patients with occlusive vascular diseases.

One hundred and fifteen male rabbits and three dogs were used in the experiments. Injections were made as follows: 1. Organisms were injected intravenously into thirteen rabbits. 2. Organisms were injected into the femoral muscles adjacent to the femoral vessels in forty-two rabbits. 3. Portions of blood vessels of human beings were embedded adjacent to the femoral vessels in twenty-nine rabbits and one dog. 4. Vessels that had been previously shown to contain pleomorphic streptococci were embedded in the pulp cavities of teeth of two dogs. 5. Micro-organisms obtained from foci (teeth, tonsils, nasopharynx and prostate gland) were injected intravenously into thirty-one rabbits.

Thirteen white male rabbits were given intravenous injections for three successive days with from 5 to 12 cc. of a culture of pleomorphic streptococci in dextrose brain broth (table 4). These cultures were obtained from arteries and veins of seven patients. They represented vessels obtained at biopsy from four patients with thrombo-angiitis obliterans, vessels from amputated extremities of two patients with

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6. Horton, B. T., and Brown, G. E.: *Ann. Int. Med.* 5:613, 1931.

thrombo-angiitis obliterans, and vessels from an amputated extremity of one patient with arteriosclerosis. Six rabbits died at the end of from four to fifty-seven days, and seven were chloroformed at the end of from three to seventy-five days. Intimal proliferation and thrombosis

TABLE 4.—*Summary of Results on Experimental Animals*

Method of Producing Lesions	Patients from Whom Material Was Obtained	Diagnosis			Source of Material		Rabbits Used	Results and Comment
		Thrombo-Angiitis Obliterans	Arterio-sclerosis		Biopsy	Amputation		
Pleomorphic streptococci injected intravenously into veins of ears of rabbits	7	6	1		3	4	13	Sections of femoral vessels from 2 rabbits showed thrombosis and intimal proliferations. Pleomorphic streptococci isolated from an acutely inflamed vein of a patient with thrombo-angiitis obliterans had been injected
Pleomorphic streptococci placed adjacent to femoral vessels	15	11	4		5	10	42	Sections of femoral vessels from 10 rabbits showed thrombosis and intimal proliferation. Pleomorphic streptococci isolated from vessels of patients with thrombo-angiitis obliterans had been injected into seven rabbits, and streptococci from patients with arteriosclerosis had been injected into three
Arteries and veins placed adjacent to femoral vessels	16	16	0		9	7	29	Sections of femoral vessels from 7 rabbits showed thrombosis and intimal proliferation, and trophic changes and dry gangrene developed in toes of hind feet of one rabbit
Green-producing streptococci, indifferent streptococci, diplococci and Micrococcus catarrhalis injected intravenously	5	5	0		2 teeth 2 tonsils 1 swab from nasopharynx		9	Negative; intimal proliferation and thrombosis not produced
	2	0	2		1 tonsil 1 gangrenous toe		2	
Washings from swab of ulcer, green-producing streptococci and Micrococcus catarrhalis injected adjacent to femoral vessels	1	1	0		Ulcer of foot		1	
	1	1	0		Tonsil		1	
Tonsil placed adjacent to femoral vessels	1	1	0		Tonsil		1	
Green-producing streptococci and Micrococcus catarrhalis injected into testes	1	1	0		Nasopharynx		1	
Micro-organisms injected intravenously	13	13	0		Prostate gland		16	

took place in the deep veins of the extremities of two rabbits. These two rabbits had been given intravenous injections of streptococci that had been isolated from an acutely inflamed vein of a patient having thrombo-angiitis obliterans. Microscopic sections (stained by Gram's method modified by Rosenow) of arteries and veins from the extremities of seven rabbits did not show micro-organisms. Cultures

obtained from the blood of the heart, the kidneys and the knee joints, were negative except in two rabbits. One rabbit (given organisms isolated from an acutely inflamed vein of a subject with thrombo-angiitis obliterans) lived four days, and positive cultures of pleomorphic streptococci were obtained from the blood of the heart, the joints and the kidneys. The blood of the heart from another rabbit on the sixteenth day contained organisms morphologically similar to those that had been injected previously. The organisms were originally isolated from the vessels of an amputated extremity of a patient with thrombo-angiitis obliterans. All cultures were incubated for thirty days or longer at 37 C.

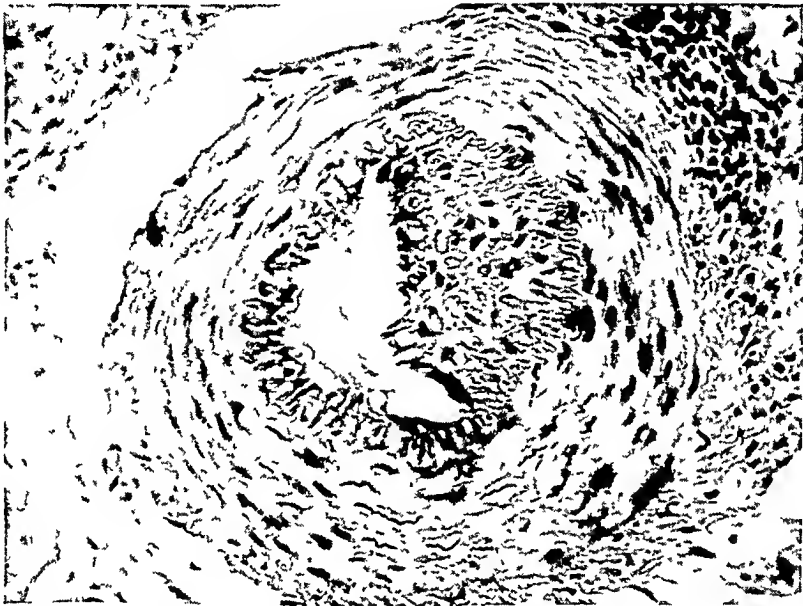


Fig. 2.—Cross-section of a small artery from the femoral group of muscles of a rabbit showing proliferation of the intima and infiltration of the adventitia by round cells;  $\times 250$ . Pleomorphic streptococci obtained from arteries and veins of an amputated extremity of a man with thrombo-angiitis obliterans (case 10, table 1) had been injected at a site adjacent to the femoral vessels. Local reaction had not developed at the site of injection. The rabbit had been killed sixteen months later.

Forty-two rabbits were inoculated with pleomorphic streptococci adjacent to the femoral vessels. The streptococci were obtained from fifteen patients, eleven of whom had thrombo-angiitis obliterans and four arteriosclerosis (table 4). Twenty-three of the forty-two rabbits lived from three to one hundred and ninety-eight days, and the remaining nineteen were killed at irregular intervals of from two to four hundred and eighty-nine days. Intimal proliferation and thrombosis took place in the femoral vessels of ten rabbits. Organisms from three specimens obtained on amputation and from three obtained at biopsy

from six patients with thrombo-angiitis obliterans were injected into seven of these rabbits. Three of the rabbits were given injections of organisms obtained from amputated extremities of patients with arteriosclerosis. Essentially the same lesions were observed following the injection of organisms obtained from patients with thrombo-angiitis obliterans as following the injection from those patients with arteriosclerosis (figs. 2, 3 and 4). Gram-positive diplococci were found in the intima, media or adventitia of the femoral vessels of eight rabbits, and in four additional rabbits intimal proliferation and thrombosis were not present. Cultures from the blood of the heart, joints, kidneys and prostate gland were all negative, except for an occasional contamination



Fig. 3.—Cross-section of a femoral artery from a rabbit;  $\times 100$ . It shows proliferation of the intima. Pleomorphic streptococci isolated from arteries and veins of an amputated extremity of a patient with arteriosclerosis (case 4, table 2) had been injected at a site adjacent to the vessel, and a local abscess had developed at the point of injection. The rabbit had been killed on the twentieth day.

with staphylococci or with gram-negative bacilli. The cultures were incubated from twenty-six to one hundred and eighty-six days.

Segments of arteries and veins from sixteen human beings with thrombo-angiitis obliterans were embedded adjacent to the femoral vessels in the muscles of the thighs of twenty-nine rabbits and one dog (table 4). Vessels obtained at biopsy were embedded in nine rabbits and one dog, and vessels from amputated extremities were embedded in twenty rabbits. The fourteen rabbits that died lived from four to one hundred and sixty-eight days. The fifteen rabbits that were killed lived from forty to one hundred and eighty-nine days. At necropsy,



Fig. 4.—Cross-section of femoral artery from a rabbit;  $\times 85$ . It shows an organized occluding thrombus surrounded by leukocytic infiltration. Pleomorphic streptococci obtained from arteries and veins of an amputated extremity of a patient with arteriosclerosis (case 4, table 2) had been injected at a site adjacent to this vessel. Local reaction had not developed. The rabbit had died fifteen days later.

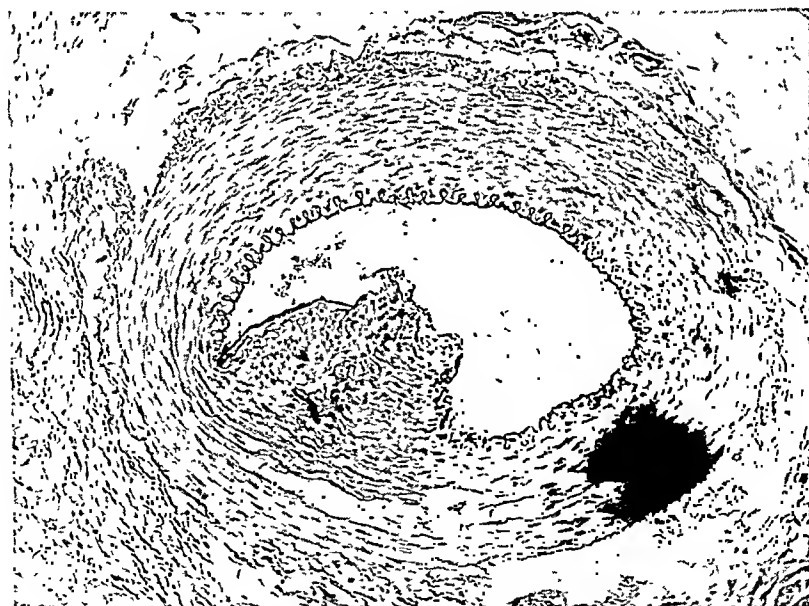


Fig. 5.—Cross-section of femoral artery of a rabbit;  $\times 85$ . It shows proliferation of the intima. A small segment of an artery obtained from a patient with thrombo-angiitis obliterans (case 27, table 1) had been embedded adjacent to the femoral artery. Local reaction had not developed. The rabbit had been killed at the end of one hundred and sixty-four days.

cultures obtained from the blood of the heart, joints, kidneys and prostate gland were negative after incubation for from twenty-six to seventy-five days. Micro-organisms resembling those previously described were found in the wall of, and in the tissue surrounding, the femoral veins and arteries of seven animals. The results of the experiment in the dog were negative. Intimal proliferation and thrombosis took place in the femoral vessels of seven rabbits (figs. 5 and 6), and in one rabbit trophic changes and dry gangrene were observed in the toes of the hind feet.

An acutely inflamed vein (fig. 7) obtained at biopsy from a patient with thrombo-angiitis obliterans (case 9, table 1) was embedded adjacent to the right femoral vessels in the right thigh, of a rabbit,

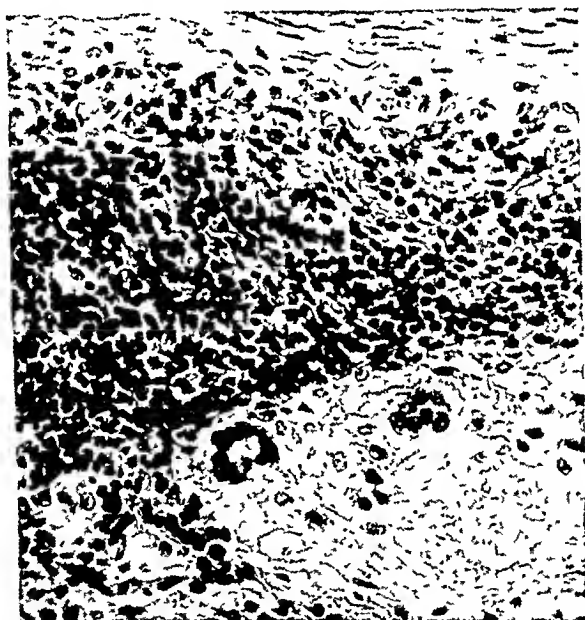


Fig. 6.—Adventitial coat of femoral artery from a rabbit, showing infiltration by round cells, with the formation of giant cells. An acutely inflamed vein obtained at biopsy from a patient with thrombo-angiitis obliterans (case 22, table 1) had been embedded in a position adjacent to the femoral artery. The rabbit had died fifty-nine days later. Local reaction had not developed at the site of the embedded tissue.

May 18, 1929. The other extremities were not disturbed. Gangrene of the toes of both hind feet had developed by June 15, 1929, and the surface temperature of the hind feet decreased markedly (fig. 8). The front feet remained normal. From a clinical standpoint, the gangrene of the toes suggested that observed in human beings with thrombo-angiitis obliterans. The rabbit was killed on June 27, 1929, and a complete postmortem examination was carried out. The femoral arteries and veins, the popliteal arteries and veins and the anterior and posterior arteries and veins were normal, but characteristic occlusive

vascular lesions, such as were observed in the femoral vessels of the other rabbits, were found in the small arterioles of the hind feet. The arteries and arterioles in the fore extremities were normal. Arteriosclerotic changes were not observed in any of the vessels. Streptococci were isolated by Rosenow from the vessels of the animal, which appeared to be identical with those isolated from and demonstrated in a section of the acutely inflamed vein of the patient.

Six rabbits were given intracerebral injections of the streptococci obtained from the vessels of the rabbit, and in one of the six thrombosis of the vessels of the brain was found. Four rabbits were given intramuscular injections in the extremities, and in one rabbit thrombosis

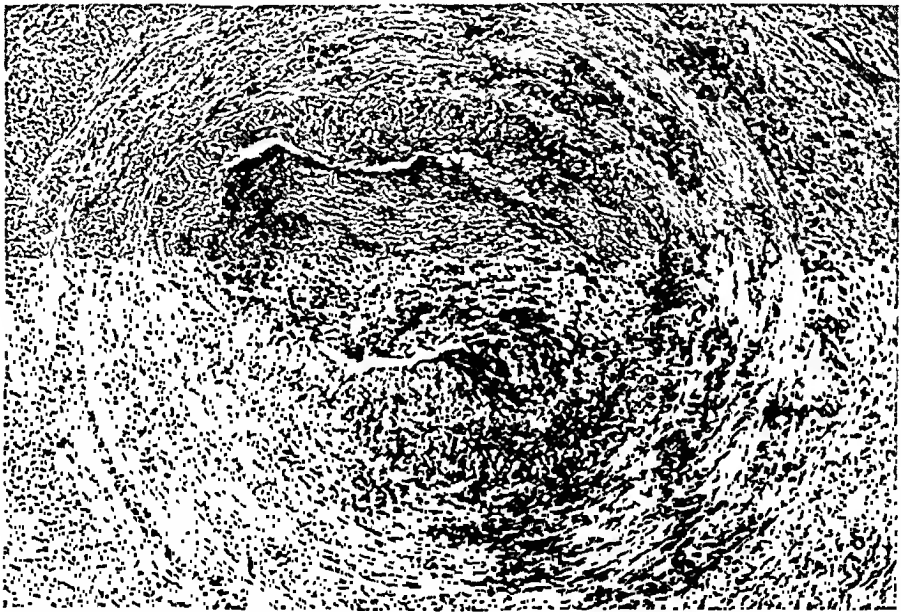


Fig. 7.—Cross-section of an acutely thrombosed vein obtained at biopsy from a patient with thrombo-angiitis obliterans (case 9, table 1). Diffuse chronic inflammatory reaction may be noted throughout the entire wall. Diplococci were found in the wall of the vein.

was found. In three rabbits that were given intravenous injections, results were negative.

Under anesthesia with iso-amyl-ethyl barbituric acid (amytal), the lower cuspid teeth of two dogs were cut off at the line of the gums, and the pulp cavities were cleaned out. A small portion of an acutely inflamed vein in which pleomorphic streptococci had been demonstrated seven days after incubation at 37 C. was obtained at biopsy from a patient with thrombo-angiitis obliterans. This was placed in the pulp cavity of one dog. Five tenths of a cubic centimeter of sediment from 150 cc. of dextrose brain broth containing green-producing streptococci that had been previously isolated from a vein of an amputated leg of a

patient with thrombo-angiitis obliterans (case 10, table 1) was injected into the pulp cavity of the second dog, and the cavities were closed with amalgam fillings. These dogs were observed over a period of two years. Clinical evidence of occlusive vascular disease in the extremities did not develop. The arterial pulsations in the extremities remained normal during this period, and the appearance of the devitalized teeth was not changed. The dogs were not killed for further study.

Since it seems to be well established that foci of infection play a part in the production of many diseases, it was thought advisable to make cultures from various foci of infection in patients with occlusive vascular diseases, and to inject the cultures into rabbits (table 4). Green-producing streptococci were the organisms predominating. Cultures containing green-producing streptococci obtained from apexes of teeth of two patients with thrombo-angiitis obliterans were injected intravenously into six rabbits; streptococci were obtained in culture

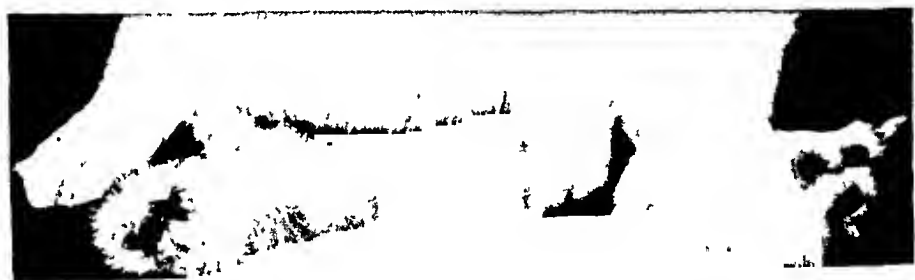


Fig. 8.—Trophic changes and dry gangrene in the toes of both hind extremities of a male rabbit. An acutely inflamed vein (fig. 7) obtained at biopsy from a man having thrombo-angiitis obliterans had been embedded adjacent to the right femoral vessels.

from the kidney of one rabbit and from the blood of the heart of another. Green-producing streptococci and *Micrococcus catarrhalis* were obtained in culture from the tonsils of three patients with thrombo-angiitis obliterans. Two rabbits were given intravenous injections, and a third rabbit was given injections in the muscles of the thigh, adjacent to the femoral vessels; the results were negative. The tonsil from a patient with thrombo-angiitis obliterans was embedded between the muscles of the thigh of a rabbit, adjacent to the femoral vessels; the results were negative. Cultures were made from the nasopharynges of two patients with thrombo-angiitis obliterans. One culture was injected into the testis of a rabbit, and the other was injected intravenously; the results were negative. The washings of a swab from an ulcer on the foot of a patient with thrombo-angiitis obliterans were injected adjacent to the femoral vessels of one rabbit; this yielded negative results. Cultures of diplococci from a gangrenous



toe and of indifferent streptococci and *Micrococcus catarrhalis* from tonsils of patients with arteriosclerosis were injected intravenously into two rabbits. The results were negative, except that diplococci similar to those previously injected were isolated from the bile and kidney of one rabbit. The fourteen rabbits that died lived from four to ninety-six days. One rabbit was killed on the sixteenth day. Sections of the femoral vessels did not contain organisms.

Material was collected from the prostate glands of thirteen patients with thrombo-angiitis obliterans and injected into sixteen rabbits (table 4), in part, as follows: Seven rabbits were given intravenous injections with cultures containing green-producing streptococci associated with either staphylococci or *Micrococcus catarrhalis*. Two rabbits were given indifferent streptococci. One rabbit was given an injection of 2 cc. of secretion from the prostate gland mixed with gelatin-Locke solution, and two were given injections of green-producing streptococci and *Micrococcus catarrhalis* in the testis. Thrombosis was not observed, and the sixteen experiments yielded negative results, except for the isolation of green-producing streptococci from the blood of the hearts of two rabbits and from the blood of the heart, liver, kidneys and prostate gland of another rabbit. The twelve rabbits that died lived from one day to two months. The four rabbits that were killed lived from five to twenty-three days.

*Comment.*—It is obvious that the pathologic lesions found in the vessels of these rabbits were similar to, if not identical with, those found in patients with thrombo-angiitis obliterans, and represent, so far as we are aware, the first lesions of this type that have been produced in animals. Rosenow stated that he has never seen these lesions occur spontaneously in rabbits. It is a question whether the lesion in thrombo-angiitis obliterans is a distinct entity, and, until this is settled, the results of experiments on animals must not be held to be conclusive. We obtained the best results either by injection of the pleomorphic streptococci adjacent to the femoral vessels or by embedding vessels from human beings adjacent to the femoral vessels of experimental animals. Inconsistent results were obtained from intravenous injections of pleomorphic streptococci into these animals. This is in accord with Buerger's experiments, as he obtained positive results only when he embedded acutely inflamed superficial veins from patients with thrombo-angiitis obliterans adjacent to normal vessels of human beings who previously had had thrombo-angiitis obliterans. He obtained negative results in animals. Our study suggests that thrombo-angiitis obliterans is of infectious origin, and that the pleomorphic streptococcus may be of etiologic significance. The presence of a filtrable virus as the causative agent of thrombo-angiitis obliterans, however, must still be considered.

## SUMMARY

Acutely inflamed veins and arteries were obtained at biopsy or at amputation of extremities in thirty-four cases of thrombo-angiitis obliterans. Gram-positive, pleomorphic streptococci were obtained in pure culture in nine cases, and green-producing streptococci, in two. Arteries and veins from ten amputated extremities of patients with arteriosclerosis were cultured in a similar manner, and five cultures yielded gram-positive, pleomorphic streptococci. Cultures from normal arteries and veins of twenty-four other patients were negative.

Four different methods were used in attempting to reproduce thrombo-angiitis obliterans in experimental animals: 1. Pleomorphic streptococci were injected intravenously into thirteen rabbits. Intimal proliferation with thrombosis took place in two. Thirty-one rabbits were given injections in a similar manner with organisms obtained from extracted teeth, tonsils, the nasopharynx and secretions of the prostate gland. Negative results were obtained. 2. Pleomorphic streptococci were injected into the femoral muscles, adjacent to the femoral vessels, of forty-two rabbits. Intimal proliferation and thrombosis occurred in ten rabbits. 3. Portions of the vessels obtained from patients with thrombo-angiitis obliterans were embedded in positions adjacent to the femoral vessels of twenty-nine rabbits and one dog. Intimal proliferation and thrombosis occurred in seven rabbits, and in one rabbit trophic changes and dry gangrene developed in the toes of the hind feet. 4. Vessels that had been previously shown to contain pleomorphic streptococci were embedded into the pulp cavities of teeth of two dogs. The results were negative. The results obtained by injecting organisms at sites adjacent to the femoral vessels of rabbits and those obtained by embedding segments of vessels from human beings in positions adjacent to the femoral vessels were essentially the same (23.8 and 24.1 per cent, respectively positive). The pathologic lesions found in the vessels of these rabbits appeared to be identical with those seen in human beings with thrombo-angiitis obliterans, and represent, so far as we are aware, the first lesions of this type that have been produced in experimental animals. The study suggests that thrombo-angiitis obliterans is of infectious origin, and that the streptococcus may be of etiologic significance.

# COMBINED CONGENITAL EXSTROPHY OF THE FEMALE URINARY BLADDER AND CLOACA

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Malformations of the cloaca may be arranged in two groups, depending on the presence and the absence of an associated exstrophy of the bladder. The factors in both are essentially the same. The reports on record demonstrate that the malformations make a transitional series from a patent urogenital sinus to an exstrophy of the bladder with intestinal fistulae in the exstrophied tissues. This discussion is limited to the latter malformations. Previous reports of combined exstrophy of the cloaca and bladder deal chiefly with evidence that the deformity may have offered in explanation of exstrophy of the bladder. Here an attempt is made to correlate the significance of the malformations with the embryologic history of the lower portion of the bowel. Spina bifida, ununited symphysis pubis, epispadias, deformities of the labia and scrotum, undescended testes, ununited müllerian ducts in the female and anomalies of the kidneys and ureters are so often associated with exstrophy of the bladder with intestinal fistulae that they have been considered a regular occurrence by some authors. There are, however, examples without anomalies of the kidneys and ureters or spina bifida.

Anomalies less frequently associated include umbilical hernia, absence of the umbilical cord, retroflexion of the trunk and differences of arterial distribution.

Combined exstrophies of the cloaca and bladder have been known for some time. Förster<sup>1</sup> in 1865 stated clearly the essential features, namely, ununited pubic bones with an exstrophy of the bladder, an opening into the lower portion of the ileum placed centrally in the upper portion of the defect, absence of the colon or presence of a rudimentary colon in the form of a short blind tube with an opening below the one for the ileum, and in the female the persistence of paired and ununited müllerian ducts opening laterally and below. In various reports,<sup>2</sup> essen-

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From the Henry Baird Favill Laboratory of St. Luke's Hospital.

1. Förster: *Die Missbildungen des Menschen systematisch dargestellt*, ed. 2, Jena, F. Mauke, 1865.

2. Ahlfeld, F.: *Die Missbildungen des Menschen*, Leipzig, 1880. Sequeira, J. H.: *J. Anat. & Physiol.* **30**:362, 1896. Shattock, S. G.: *Tr. Path. Soc. London* **46**:248, 1895. Keith, A.: *Brit. M. J.* **2**:1857, 1908. Wood-Jones, F.: *J. Anat. & Physiol.* **46**:193, 1912. Johnston, T. B.: *ibid.* **48**:89, 1913. von Geldern, C. E.: *Arch. Surg.* **8**:61, 1924.

tially the same anomalous conditions are mentioned. The explanations of origin differ.

In the reports by Doran,<sup>3</sup> Bryce<sup>4</sup> and Emrys-Roberts and Patterson,<sup>5</sup> the anomalous conditions appear similar to those discussed, but differ enough to be mentioned separately later.

According to Connell,<sup>6</sup> exstrophy of the bladder was first reported in 1595 by John Schenke. The first record of this malformation in the female is by Van Horne, in 1670. Since then, the number of reports has increased rapidly, and many theories have been advanced in explanation of the defect. Förster,<sup>1</sup> in 1865, seems to have been the first to discuss exstrophy of the bladder associated with intestinal fistulae. These malformations are much less common than the uncomplicated exstrophies of the bladder, but the etiology of both probably centers on a common factor. Connell<sup>6</sup> reviewed in detail the theories of exstrophy of the bladder and classified them under three general headings, namely: (1) mechanical, (2) pathologic and (3) due to arrested development. At this time, however, three hundred years since the first case was described, no theory of etiology is universally accepted.

#### REPORT OF A CASE

A full term female infant was born at St. Luke's Hospital, in the service of Dr. H. K. Gibson, on April 16, 1931. The mother had three healthy living children. The labor was prolonged. There were no deformities of the head, chest or extremities, except a left equinovarus. There were no changes of the heart or lungs. The umbilical cord was 35 cm. long and had a broad base through which the loops of bowel were visible. Below this was a defect of the anterior abdominal wall; from the center of this defect protruded the mucosal surface of an evaginated segment of bowel. Urine and feces passed onto this surface; it became markedly reddened and the edges gangrenous. On April 19, the infant had a fever of 99.2 F.; breathing became labored, and she died the next day.

Only the essentials of the postmortem record are mentioned. The body weighed 2,585 Gm. and was 44 cm. long. There was slight general icterus. The stump of the umbilical cord was dry, and below the base was a dry, tan-brown, tough membrane 7.3 by 3 cm. A huge cleft of the abdominal wall below the umbilicus formed a triangular region 9 cm. wide and 14 cm. long over the curved surface of the distended abdomen. This mass of soft red tissue bulged because of the intra-abdominal tension and was divided into three parts. In the center, a tissue 5 cm. long and 3.5 cm. wide protruded 2.4 cm. and was covered by a glistening mucosa. It was surrounded on each side by a crescent-shaped lateral mass also covered by a smooth, glistening red mucosa. The central bulging portion contained an opening into the ileum. The red lateral masses presumably were the dorsal wall of the bladder. At the bottom of the reddened mass where the edges of the skin united

3. Doran, A.: *J. Anat. & Physiol.* **15**:226, 1881.

4. Bryce, T. H.: *J. Anat. and Physiol.* **29**:553, 1895.

5. Emrys-Roberts, E., and Patterson, A. M.: *J. Anat. & Physiol.* **40**:332, 1906.

6. Connell, F. G.: *J. A. M. A.* **36**:637, 1901.

in the perineal region was a small bifid, tonguelike mass of tissue, the genital tubercle. Extending upward and laterally the mucocutaneous margin was widened and thickened on each side by a ridge of skin representing the labia majora. An anus, proctodeal depression and spina bifida were not present. The pubic bones were widely separated.

The body was opened by a midline incision extending down to the cleft in the abdominal wall and then laterally on each side to preserve intact the structures concerned with the malformation. The abdominal wall below the umbilicus was less than 1 mm. thick and had no muscle tissue. The liver weighed 150 Gm.; the right lobe in the right anterior axillary line extended 8 cm. below the costal margin to the iliac crest. The left lobe of the liver in the midline was 9 cm. below the tip of the xyphoid process. The small bowel, distended with gas, seemed to have the usual length and was attached by a mesentery in the midline. Several centimeters of the distal portion was adherent to the visceral surface of the evaginated tissues. The small bowel reached the floor of the small pelvis, curved upward, herniated into the bulging mass previously described, and opened to the external surface in the upper portion of the protruding mass. There were two other openings in the central mass. One, 6 cm. below that for the small intestine, entered a blind segment of bowel, 1.5 cm. in diameter and 6 cm. long, attached in the midline in front of the spine by a short mesentery. It had no paired appendages; the other, 1 cm. above the latter, opened into a small appendage having the shape and size of an appendix vermiformis, 2.5 cm. long and 0.3 cm. in diameter. This was coiled along the side of the visceral surface of the bulging mass and was attached there by a thin mesenteriolum. On the external surface, 1 cm. below the inferior margin of the bulging mass, their centers 0.9 cm. from the midline, were the paired openings of cone-shaped appendages, each 4.5 cm. long, 2.5 cm. thick at the base and 0.6 cm. in diameter distally, and ending in a short coiled tortuous tube (fallopian tube). Beneath the end of each tube was a mass of soft, dark red tissue, 1.3 by 0.3 cm., which by histologic examination was identified as ovary. There was no connection between the cone-shaped structures (müllerian ducts). The colon was absent. The kidneys had no unusual features. The ureters entered the pelvis medial to the paired cone-shaped appendages, where they diverged and opened on the lateral masses of tissue 1.5 cm. above and outside of the opening leading into the paired appendages (müllerian ducts). Except for bronchopneumonia there were no other changes elsewhere in the body.

#### COMMENT

The descriptive anatomy of these combined exstrophies in the reported accounts is essentially the same. There is, however, a difference of opinion regarding the nature of the various structures in this condition. The intestinal openings on the ventral wall of the body were interpreted by Ahlfeld, Keith and Sequeira as the misplaced vitelline duct, an opinion not widely accepted. Ahlfeld thought the misplacement was due to a short umbilical cord, but this explanation does not apply to malformations with a normal umbilical cord.

Some authors considered the malformation a persistent cloaca. The embryologic history of the cloaca supports this view, in that a urogenital aperture corresponding to the upper extent of the primitive cloacal membrane prior to the separation of the cloaca into bowel and bladder

would result in such an anomaly.<sup>7</sup> Johnston<sup>2</sup> and von Geldern<sup>2</sup> attempted to clarify the matter by a histologic study of the tissues. Both identified the lateral red regions as vesical mucosa and the central portion into which the intestines open as large intestine. The mucosa of the upper opening was small intestine. Johnston stated that if a layer of bladder epithelium could be demonstrated, interposed between the normal skin below the umbilical cord and the intestinal mucosa, the cloacal nature of these structures would be established and the contention of vitelline duct participation in the anomaly refuted. Von Geldern was able to demonstrate such a layer of bladder epithelium. In the case now reported, the identification of the lateral red regions as urinary bladder was made by demonstrating ureteral openings in these tissues. The epithelium of these regions had desquamated, and the histologic preparations were, therefore, unsatisfactory. The mucosa of the central bulging mass had fine villi covered with columnar epithelium but no true valvulae conniventes. The muscular layer was well developed; although the sections were bowel wall, they had no distinguishing structures by which to differentiate between small and large intestine.

The central mass anatomically represented a large cecum, with three openings: (1) into a vermiform appendix, (2) into the small intestine at the upper portion and (3) into a blind colon at the base. Sections of the appendix and of the small and large bowel had the usual structure of these tissues. Johnston's specimen had a blind diverticulum that corresponded anatomically to the colon in the case now reported, but did not have tissues of an appendix. The blind diverticulum in Johnston's report had mucosa which, at the distal end, was like that of the large bowel and, proximally, was a transition between small and large intestine. The anomaly described by von Geldern had no structure corresponding to the large bowel, but had paired diverticula that histologically resembled large intestine. They may have been the colon and a large appendix. A structure anatomically corresponding to the colon has been reported by all authors except Emrys-Roberts and Patterson, and Bryce, and a single or bifid appendix vermiformis by all except Bryce and Doran. The histology of these structures was not recorded by the authors. An anomaly in a full-term fetus, described by von Berenberg-Gossler<sup>8</sup> has considerable significance. This fetus had an anal fold, two scrotal sacs, a rudimentary penis but no anus or urethra. The distal loop of the ileum was connected with the vertex and posterior wall of the urinary bladder by a tube (designated X). The cecum with two vermiform appendixes and a short dorsally curved colon end-

7. Arey, L. B.: *Developmental Anatomy*, Philadelphia, W. B. Saunders Company, 1926, p. 148.

8. von Berenberg-Gossler: *Anat. Hefte* 49:615, 1913.

ing blindly was continuous with this tube behind and below. Von Berenberg-Gossler concluded, like Kermauner and, later, Johnston, that the cloaca forms a much larger part of the bowel than is generally believed.

The intestinal tract originally is a straight tube. Subsequently, a marked growth in length occurs. The center of this growth activity, according to von Berenberg-Gossler, is in the cloaca, from which develop the lower portion of the ileum, the cecum, the appendix, the colon and the rectum. This conclusion seems to be supported by the anomalous anatomic relations found in the case here described and other combined exstrophies of the urinary bladder and cloaca.

#### SUMMARY

Combined congenital exstrophy of the female urinary bladder and cloaca was observed in a full-term child that lived four days after birth.

A plausible explanation for the anomaly assumes that the cecum has a direct anatomic relation with the primitive cloaca and that growth activity concerned with the formation of the lower portion of the ileum, the cecum, appendix, colon and rectum centers in the cecum.

Conversely, the theory proposing such an embryologic process for these intestinal structures is supported by the anomalous anatomic conditions found in the combined exstrophies of the urinary bladder and cloaca.

# General Review

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## THE ORIGIN AND ANTIQUITY OF SYPHILIS: THE EVIDENCE FROM DISEASED BONES

A REVIEW, WITH SOME NEW MATERIAL FROM AMERICA

HERBERT U. WILLIAMS, M.D.

BUFFALO

*Concluded from page 814*

## REVIEW OF ALL REPORTED CASES OF ANCIENT SYPHILIS OF BONE

### AMERICA

The first reference to syphilis of bone in remains of American Indians that I have found is in a brief note by Farquharson, dated 1875. In an account of the exploration of mounds near Davenport, Iowa, he said of the skeletal remains: "Evidence of the prevalence of syphilis was quite common in the form of nodes."

Shortly after this, there appeared the monograph of Dr. Joseph Jones of New Orleans, whose report was of considerable importance and excited much discussion in various parts of the world. The work of Jones will be considered farther on in the present article. Parrot's statements concerning certain skulls from Peru appeared about the same time.

In subsequent years, many other specimens of bones alleged, with more or less confidence, to be both ancient and syphilitic, have been described. For the sake of clarity it seems to me best to consider first the cases that satisfy the two criteria mentioned most completely, rather than to take them up according to their geographic distribution. The specimens in order of importance are: skulls found by Kidder at Pecos, N. M., and described by Hooton; a skull and some long bones found by Tello at Paracas, Peru, and described by Tello and Williams; a skull from the Rio Negro region in Argentina; several long bones from one skeleton found by Kroeber in the Cañete Valley in Peru, now in the Field Museum, Chicago, not previously reported on; long bones excavated by Mills and Shetrone from mounds in Ohio, some of which have, and some of which have not been, previously described; a skull collected by Dr. Joseph Jones, Big Harpeth River, Tenn.

Besides these, there are numerous skulls and long bones from points as widely separated as the middle of the United States and Argentina, many of which are important, though not so well authenticated as the first six lots of specimens.



*Skulls from New Mexico.*—At Pecos, N. M., extensive excavations of a ruined pueblo have been made by A. V. Kidder for the department of archeology, Phillips Academy, Andover, Mass., beginning in the year 1915.<sup>9</sup> The Spaniards of the expedition of Coronado (1540-1542) found this pueblo inhabited by several hundred people. A diminishing number of their descendants continued to occupy it until 1838. According to Kidder, his excavations passed through a number of superimposed strata, with which he was able to associate various types of pottery.<sup>10</sup> The lowest stratum, belonging to the earliest occupation, was dated as from 800 to 1,000 years ago (Hooton, p. 332).

An enormous amount of skeletal material was obtained by Kidder, which has been described by Hooton, including three specimens strongly suspected of being syphilitic. In Hooton's words (p. 311): ". . . all of them are definitely and indisputably prehistoric." His case 60455 I consider the best piece of evidence now extant for the existence of pre-Columbian syphilis in America. These specimens are all in the Peabody Museum, Harvard University, Cambridge, Mass. The fact that the nasal region is involved in all three cases is probably the result of coincidence; in point of time, the three belong to periods probably one or more centuries apart.

Pecos Case 60455 (skull and right femur from the level "black and white," or "glaze I," therefore very ancient [figs. 18A, 19 and 20]): According to Hooton, the skull is that of a middle-aged female. It shows well marked deformation from flattening of the posterior part of the parietal bone and the adjacent part of the occipital bone on the left side, which is common among Indian skulls. It is rather heavy, though not exceedingly so. The enamel of the teeth is considerably worn away by grinding, as is frequent in old Indian skulls; one molar in the upper jaw shows some caries; there is no notching of the two incisors remaining. There is partial destruction of the nasal bones, with subsequent healing; the vomer and turbinates are loose and in large part missing, probably lost post mortem, as frequently happens in these Indian skulls. The most characteristic lesion is shown on the frontal bone, of which the greater part is involved, only the posterior one fifth being exempt. The surface is roughened by the presence of many low, flat elevations, some small, some large, separated by irregularly running, linear depressions,

9. In 1926, during a brief visit, I saw these excavations in progress; even to an amateur it was evident that they were conducted with most scrupulous care.

10. The succession of cultures indicated by the various types of pottery is shown in the following table (Hooton, p. 10):

1. Late archaic: corrugated (little), strong blind corrugated; black on white.
2. Period of the introduction of glaze: strong and medium blind corrugated; glaze I, biscuit A.
3. Period of concentration: faint blind corrugated; glaze II and III, biscuit B.
4. Late prehistoric (?) 1600: featureless black; glaze IV, glaze V, biscuit B.
5. Early historic, 1600-1680: striated black, late glaze V, beginning modern (?).
6. Late period, 1680-1840: striated black (?), plain red, polished black, modern.

frequently stellate and corresponding closely with the description given by Virchow for typical syphilitic lesions of bone (fig. 19). On both sides, the process extends downward as far as the articulation with the malar bone and nearly or quite to the orbit and nasal bones. On the left, near the junction with the malar bone, is a depression with slightly elevated edges radiating from it, indicating a lesion nearly, but not completely, healed. On the right, just in front of the middle, are three more depressions of the same character. The most conspicuous lesion is an area just to the left of the middle, roughly rectangular in form, about 4 by 3 cm., where the external table and diploe have evidently been destroyed by necrosis, while the

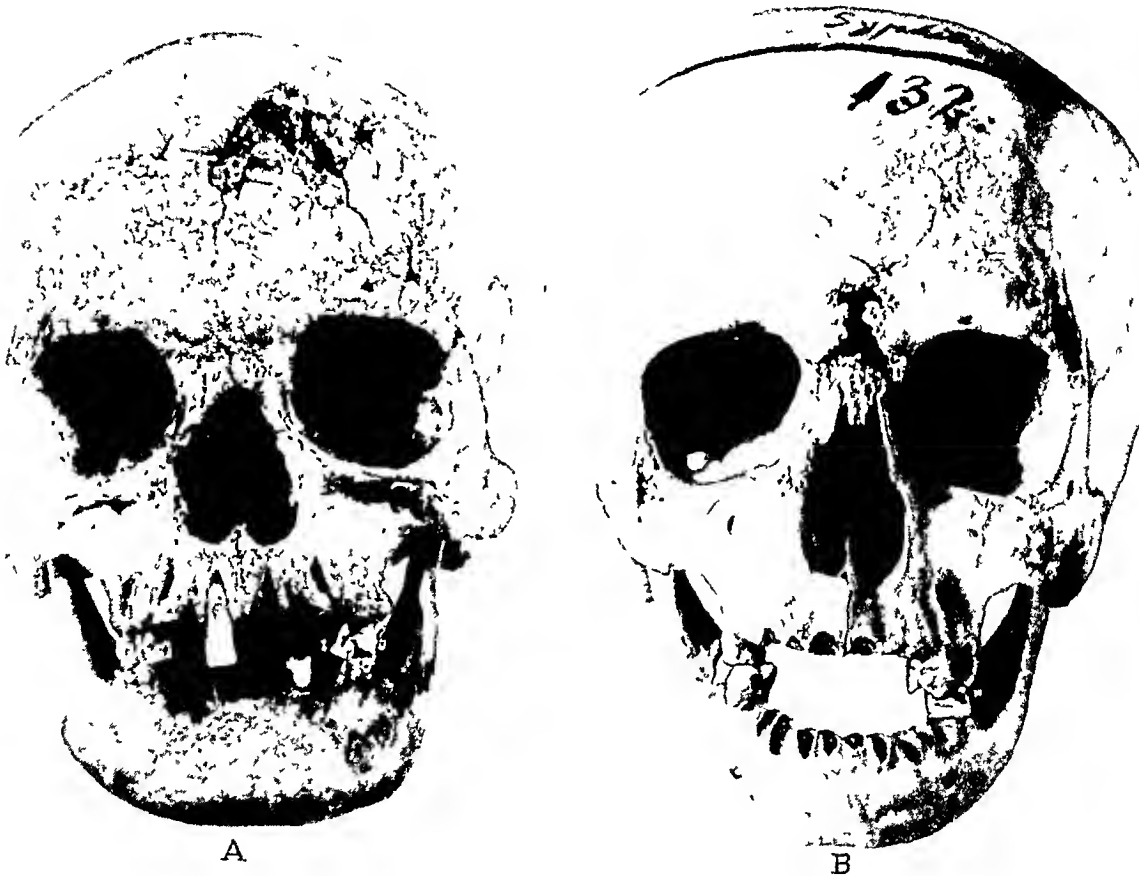


Fig. 18.—*A*, Pecos case 60455. The photograph was furnished by Prof. E. A. Hooton, Peabody Museum, Cambridge, Mass. *B*, the skull in a case of congenital syphilis, Prague. The photograph was furnished by Prof. A. Ghon.

borders are elevated and partly healed. The central part of this area still contains what may be the remnants of a sequestrum. The posterior part of the edges of this area shows fine lines of new-formed bone running in the direction of radii drawn from the center of the whole area. The inner surface of the skull apparently is slightly roughened over a region corresponding to the same area. The right parietal bone has an area, roughly 5 cm. square, but of irregular form, extending from the parietal eminence nearly to the coronal suture, where there are a little

caries and a number of small, stellate scars. Over the left parietal eminence is a smaller area of the same sort, from 2 to 3 cm. in diameter.

I have examined this specimen several times, and in my opinion, if it is possible to make a diagnosis from a dried bone without other evidence, this must be called a syphilitic skull. It so closely corresponds with an old skull in the museum of the German University at Prague on which a diagnosis of congenital syphilis was made (autopsy in 1874 probably by Preitz; no history; girl of 15) that a photograph is introduced for comparison (fig. 18 *B*).

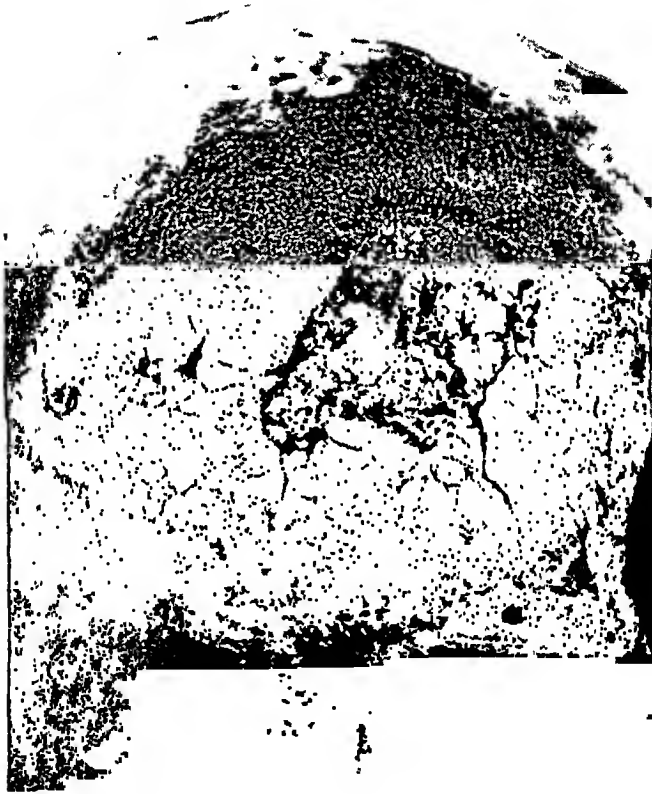


Fig. 19.—Detail from Pecos case 60455.

One has, however, some more evidence in case 60455 in the shape of the right femur (fig. 20), which shows on the inner border a well marked thickening, partly in the lower half of the shaft but rather more in the upper half. Its surface is smooth and rounded. The roentgenogram shows plainly that there is a rather dense bony growth from the periosteum, encroaching slightly on the medullary canal below. A small piece was taken from the posterior surface and sectioned. The microscope showed that it has the structure of a periosteal osteophyte. There are none of the marks of periostitis with osteomyelitis, and the location would be an unusual one for periostitis due to injury. The femur, therefore, makes valuable confirmatory evidence for the syphilitic nature of the disease shown on the skull.

Pecos Case 59864 (skull from the level "glaze II," according to Hooton, from a middle-aged woman [fig. 21]): An ulcerative inflammation has destroyed the

lower portion of the nasal bones and the borders of the apertures. There was perforation of the alveolar borders through the alveoli of the right canine and the lateral incisors. The roof of the palate is cicatrized. There are areas of thickening on the frontal bone, the malars and the orbital walls. There are areas of erosion on the left side of the frontal bone, coming down to the supra-orbital ridge, with irregular

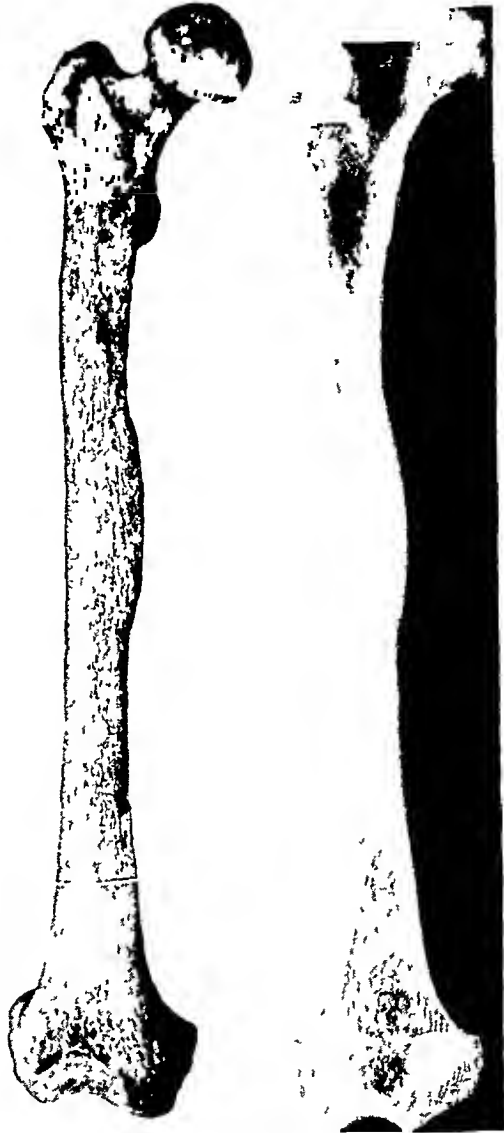


Fig 20.—Pecos case 60455, right femur (original photograph). The roentgenogram was made by Dr. E. C. König, of the Buffalo General Hospital.

linear scars like those seen in syphilis of the cranium, of which this is in all probability a case.

Pecos Case 59814 (from the level "glaze III" [figs. 22 and 23]): This is a fragment of a skull; according to Hooton, it is from the skull of a young woman. As described by Hooton: "The roof of the palate shows evidence of inflammation and a pathological deposition of bone. The posterior third of the palate is

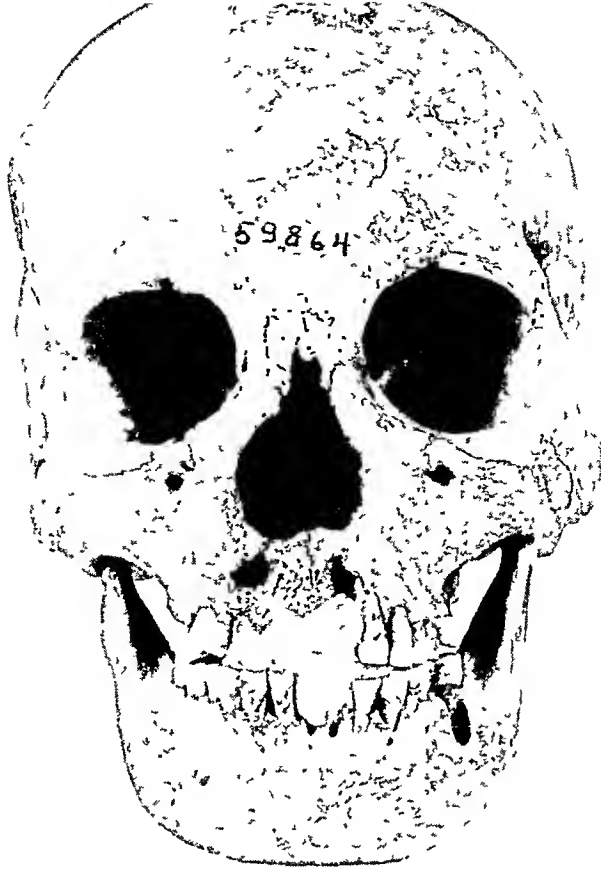


Fig 21.—Pecos case 59864. The photographs for figures 21, 22 and 23 were furnished by Prof. E. A. Hooton, Peabody Museum, Cambridge, Mass.



Fig. 22.—Pecos case 59814, perforation and inflammatory changes of the palate.

perforated by a hole almost square in shape, but with corners rounded and with the edges bevelled and cicatrized. This hole is about 2 cm. square. The nasal orifice is a round, cicatrized hole of about the same size as the perforation through the hard palate, with which it is continuous. The borders of the nasal aperture have been covered with a thick, bony deposit which is bevelled inward, and the lower halves of the nasal bones are involved in this cicatrix." The diagnosis in this case is puzzling and in my opinion cannot be made with certainty. The lesion about the nose suggests to me traumatism as a cause, but the perforation of the palate seems very significant on account of the frequency of this lesion in syphilis.



Fig 23—Pecos case 59814, cicatrized nasal aperture.

*Skull and Long Bones from Paracas, Peru.*—The peninsula of Paracas protrudes as a kind of point into the Pacific Ocean, 18 kilometers south of the port of Pisco, which again is about 200 kilometers south of Callao and Lima. The locality is one of exceptional desolation even on that arid and forbidding coast.

Dr. Julio C. Tello, then curator of the Peruvian Archeological Museum at Lima, made explorations that disclosed the presence of curious bottle-shaped tombs, buried below the sand and excavated in a hard clay or shale (Tello, 1929). The tombs contained numerous mum-

mified bodies wrapped in beautifully embroidered cloths. Many of the skulls had been trephined. The characters of the pottery, implements and ceremonial objects led Tello to conclude that the burials antedated the well known Nasca period; others regarded them as indicating a variety of early Nasca culture. In any case, they were much earlier than the Inca period, which began about 1200 A. D. Tello repeatedly found evidence that when the tombs became filled, some of the older bodies were removed to make room for the more recent ones. The bodies thus

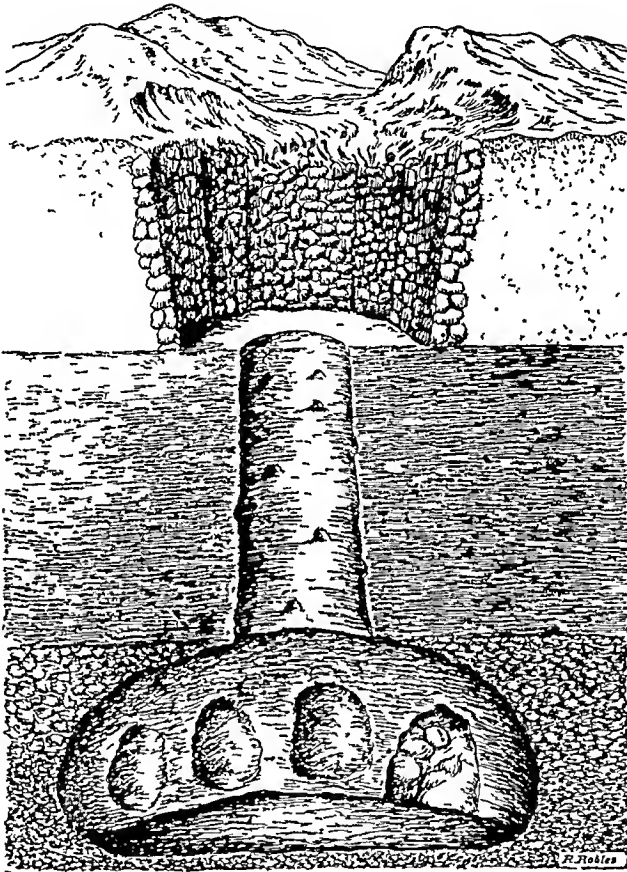


Fig. 24.—A diagram of tomb 5, Paracas, Peru, showing the vestibule above, the cavern below, and the tube that connects the two. This and seven other illustrations of this case are from an article of Tello and Williams (*Ann. M. Hist.* 2:519, 1930).

removed were buried around the entrance to the tomb; the specimens of interest to the readers of this paper seemed to belong in the latter category; they were found just outside of the tomb. The condition of affairs is shown clearly in Tello's diagram of tomb 5, which contained forty-eight bodies (fig. 24). The tomb proper opened to the exterior by a tube 1.66 meters in height, with a diameter of 1 meter at the bottom, the upper opening being covered by bones of whale, skins and

mat. The upper opening was surrounded by an enclosure called the vestibule, which was lined with stones and resembled a shallow well. Several skeletons and some other bones were found in the vestibule and in the sand just above it. Two of the diseased bones, a left tibia and a left humerus (case 12-7509), were found in the vestibule. Removal of the sand in the places contiguous to the cavern disclosed other diseased bones, among them the cranium, the two femurs and the ulna.

The skulls found in the Paracas tombs generally showed a peculiar type of artificial deformation called by Tello "cuneiform," produced by pressure on the frontal and occipital bones. Tello found some bodies with the apparatus for deformation actually in position, in principle like

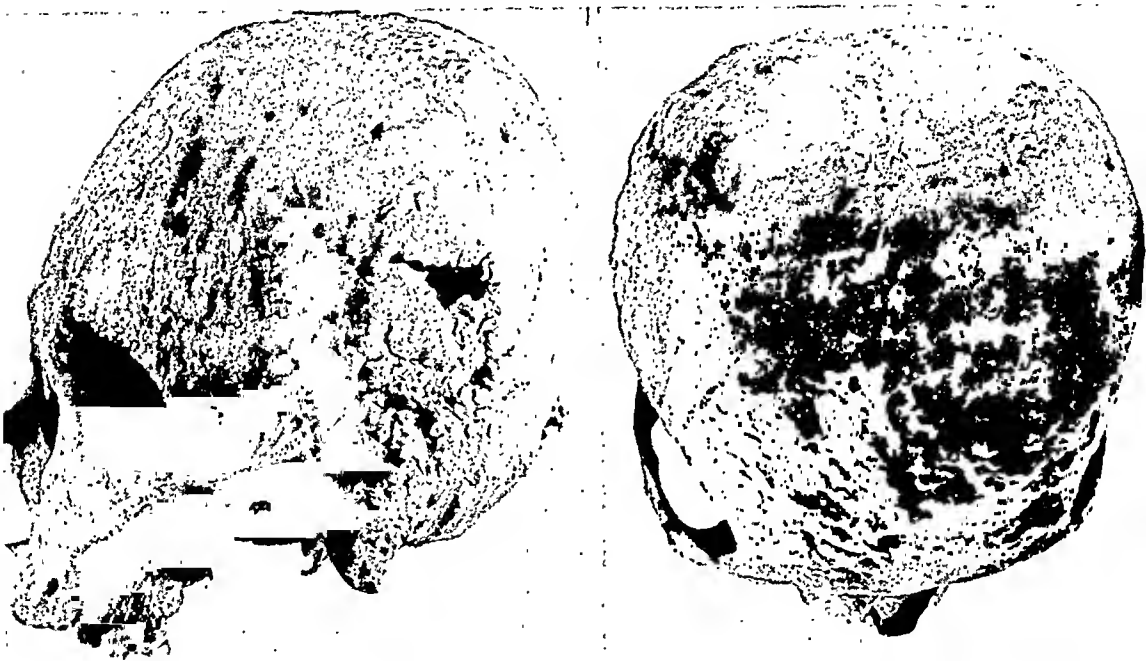


Fig. 25.—Left, a lateral view of the ancient skull from Paracas, Peru, case 12-7509; right, frontal region of same skull. The photograph was made by the American Museum of Natural History through the courtesy of Dr. H. L. Shapiro.

the apparatus advertised by modern "beauty doctors." The skull (12-7509) showed the same type of deformation as in those found in the tombs. This and the long bones presented a dark yellowish-brown patina like old ivory. The cultural objects, among them a plate of gold in the form of a five-pointed star, found with the bones outside of the tomb were of the same kind as those found within the tomb; there were no cultural objects belonging to a later period. While the possibility that the bones under discussion were introduced through an intrusive burial must be conceded, the facts stated indicate that they were of the same age as the bodies within the tomb. The wild and lonely nature of the surroundings also made a recent intrusive burial unlikely.



In November, 1929, I visited Paracas in company with Tello. It was apparent to me that only the person who actually conducted the original excavations was in a position to decide as to the antiquity of the bones to be described. Concerning this, Tello had not the slightest doubt. He is the most experienced archeologist in Peru, having made archeologic exploration in many parts of his country, both in the Andes and along the coast, his life work as is well known to other archeologists and anthropologists.



Fig 26—Detail of skull from Paracas, case 12-7509.

The specimens are described in an article by Tello and myself. The illustrations and the descriptions of the bones here are copied from that article. It is not certain that the skull and the long bones came from the same skeleton, but they probably did so. The skull is syphilitic, as far as it is possible to make the diagnosis on a dried specimen without the clinical history.

Skull from Paracas (figs 25 and 26). The region involved by the disease includes the frontal bone, both parietal bones, the occipital bone and both mastoids. The involvement is most marked on the left side, behind and below, that is, the lower posterior angle of the left parietal bone and the adjacent mastoid bone.

Nearly the whole frontal bone from the ridges of the eyebrows back is affected. The surface presents low elevations, varying in size, but about 1 cm. in diameter, between which are depressions, which, especially on the right side, are frequently linear, sometimes stellate.

The right parietal bone is affected in the same way as the frontal bone, but less notably. Just below the parietal eminence are two minute openings into the interior of the skull; each is situated at the base of a small, depressed scar; they were probably produced by disease. Another small opening a little farther back was probably made post mortem.

The disease appears to have extended on to the right mastoid from the adjacent parietal and occipital bones; the changes here are not marked.

The anterior half of the occipital bone is involved; the irregularity of the surface is not great. At about the middle of the area of involvement occurs a depression, 1.25 cm. long by 2 cm. broad, approximately rectangular in form; it is due to erosion of the external table, probably produced by active ulceration, but possibly formed post mortem.

The surface of the anterior half of the left parietal bone is only slightly irregular; the posterior half exhibits severe involvement. Just behind the parietal eminence is an area of ivory-like, irregular thickening, about 4 cm. long by 3 cm. broad. In the lower posterior half of the parietal bone occur four openings into the interior of the skull. Three of these openings are minute, being 1 to 4 mm. in diameter. All occur at the bottoms of depressed scars. None of them could be due to postmortem erosion. The fourth and lowest opening is larger. Its form is very irregular, something like an ax with a short handle; its greatest length is about 28 mm.; its greatest breadth about 10 mm. Its edges are thin, but rounded. There is no evidence of injury or fracture. It bears no resemblance to the results produced by trepanation, as could be determined by comparison with many admirable examples of trepanation done by the ancient Peruvians in the large collections under Tello's direction. It is evidently the result of ulceration that has healed. Below this opening are five small areas of active ulceration, two on the parietal bone, two on the mastoid bone and one at the junction of the parietal and mastoid bones. Over the lower and posterior part of the parietal bone are about nine irregular, short, linear scars, some of them stellate, corresponding closely in form to the lesion described by Virchow as pathognomonic of syphilis.

The inner surface of the skull is smooth. There is no indication of fracture. The skull is rather heavy. It is moderately flattened anteroposteriorly. The sutures are nearly obliterated. The lower jaw is missing. In the upper jaw, the second right molar, the third left molar and the stump of the first left molar are the only teeth present. The second bicuspid and first molar on the right were lost during life, as was shown by atrophy of the alveolar process. The skull was evidently that of an elderly person.

Long Bones from Paracas (fig. 27): The long bones are two femurs and the left tibia, humerus and ulna.

The right femur is 40 cm. long. It shows well marked thickening at about its middle third on the anterior and outer aspect. The area of thickening is smooth and is evidently a new growth from the periosteum. The left femur is 39.5 cm. long. It is thickened over a part of its anterior and outer aspect, beginning below the great trochanter and extending over about the upper fourth. There is thickening also in front, extending to a point below the middle. The area of thickening is mostly smooth, though it shows some small openings and short vertical grooves. The growth is evidently periosteal.

The angles made by the necks of the femurs with the shafts indicate that the femurs came from an elderly person.

The left tibia is 33.5 cm. long. It presents the most marked involvement of any of the long bones, almost all of it being affected except the region just below the head. It is enlarged, the circumference at the junction of the upper and middle thirds being nearly 10 cm. The surface is slightly nodular and is roughened by having many punctate openings and short vertical grooves.



Fig. 27.—Long bones, case 12-7509 (photograph from American Museum of Natural History, New York).

The left humerus is 27.5 cm. long. It is thickened moderately in its upper half, especially in the second quarter from above, where there are many small openings, partly longitudinal. It is not noticeably heavy.

The left ulna is heavy, in spite of the fact that a small portion of the lower end is broken off. The portion remaining is 21 cm. in length. It is thickened throughout its circumference and throughout its length. The surface is slightly nodular and is made still more irregular by many small openings, partly longitudinal,

shown especially at the upper and lower ends. It bears considerable resemblance to bones seen in cases of acute periostitis and osteomyelitis.

The absence of sinuses and sequestrums in any of these long bones may be noted as being in favor of the diagnosis of syphilis and against that of periostitis and osteomyelitis.

An admirable roentgenogram of the long bones was made for us by Dr. Oscar Soto of the Loayza Hospital, Lima (fig. 28). The new growth of periosteal bone

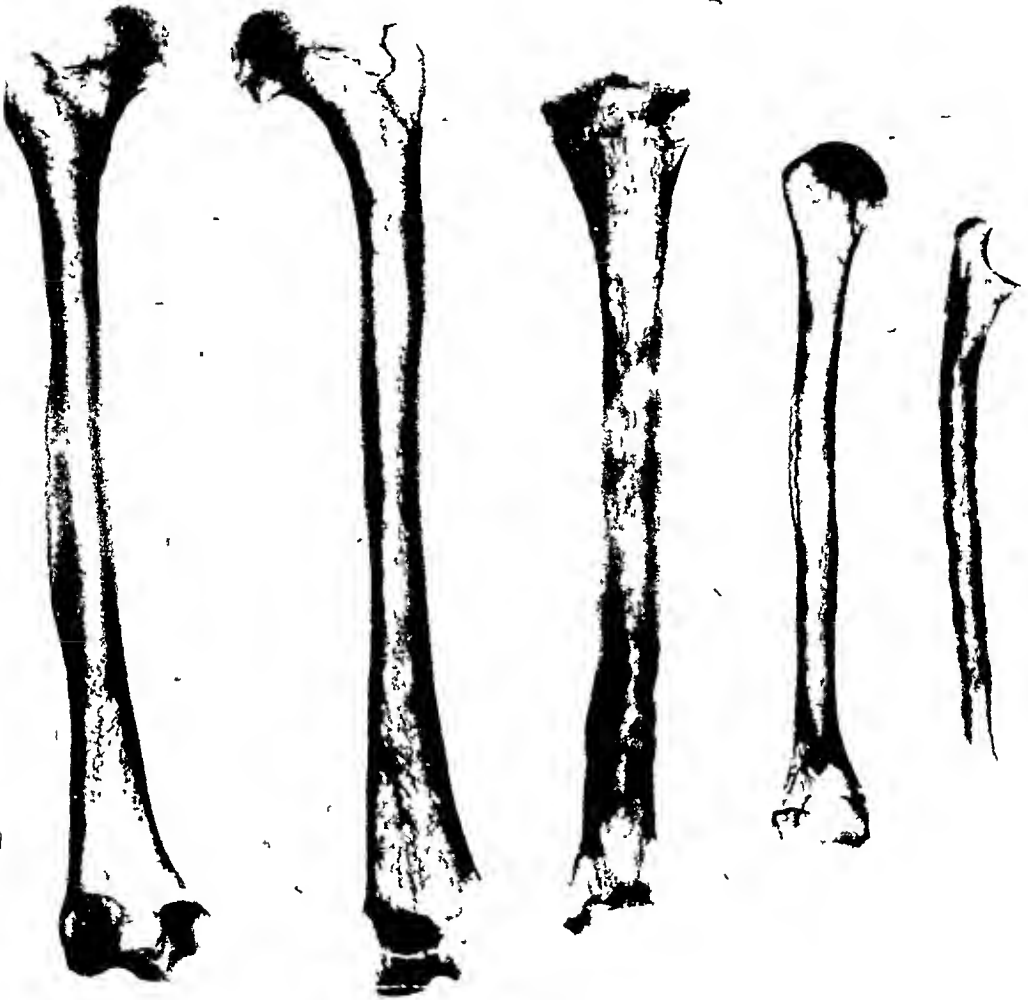


Fig. 28—Long bones, case 12-7509. The roentgenogram reproduced here was one of the best examples of the roentgenologist's art that I have seen; it has suffered greatly in being reduced in size and in the loss of detail in the process of photo-engraving. It was made by Dr. Oscar Soto, Loayza Hospital, Lima, Peru.

in all of the bones is plainly shown. There is also some encroachment on the medullary canal. The delicacy with which the normal framework of the bones appears in the roentgenogram, especially at the ends of the femurs, is probably due to removal of some of the calcium salts by natural agencies post mortem. In having small pieces of the bones decalcified to make sections for study with the microscope, referred to in a subsequent paragraph, it was remarked that decalcification

with 5 per cent nitric acid was unusually easy and rapid. The x-ray picture is consistent with syphilis; it could have been produced by chronic periostitis and osteomyelitis; it does not resemble that seen in osteitis deformans (Paget's disease).

For examination in sections, the right femur and left tibia were sawed through vertically (fig. 29 *A*). The new growth of periosteal bone on the surface is plainly shown. The narrowing of the medullary part of the bone is especially marked in the tibia. The evidence given by the roentgenogram is therefore confirmed and amplified.

After the right femur and left tibia had been sawed through, pieces were removed from the most thickened parts. In 5 per cent nitric acid, they were easily and rapidly decalcified. Sections were made after fixation in formaldehyde and embedding in celloidin. These were stained with hematoxylin and eosin; the hematoxylin was practically without effect. The outer surface stained more deeply with eosin than the inner parts (fig. 29, *B* (femur) and *C* (tibia)). The cavities occupied by the bone cells were visible, but not distinct. Foreign material was plentiful, especially in or near the outer surfaces. It consisted of dark particles, evidently soil, threadlike masses and small round bodies that were derived from some unidentified fungus, and large, generally rounded, yellow masses that could not be identified.

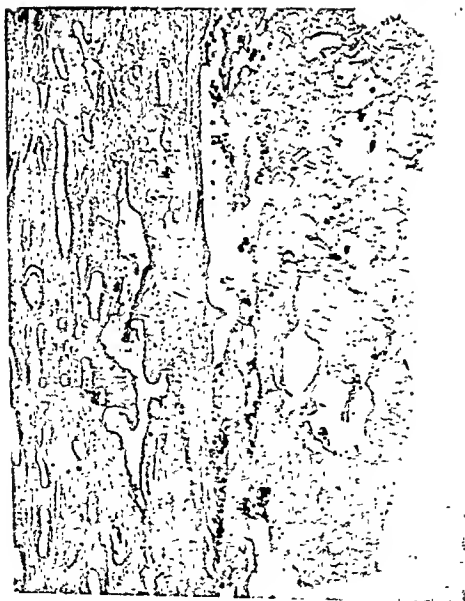
The sections show that both bones consist of layers of lamellae, which are deposited very irregularly near the outer surfaces. Both show numerous cavities of considerable size, running in general parallel with the long axes and with the outer surfaces. The line between the new-formed periosteal bone and the underlying bone is well marked in the section from the femur; in the section from the tibia, the line is less distinct. In general, the structure agrees with the descriptions given for syphilitic long bones. The large size of the spaces appears to me to indicate the presence of osteitis as well as of periostitis; this view is in agreement with the encroachment on the medullary part of the bone shown in the roentgenograms and in the longitudinal sections. Haversian systems, poorly developed, appear in both sections in small numbers near the outer surfaces. In my opinion, the sections from the Paracas bones indicate the presence of an osteoperiostitis that was probably caused by syphilis, though possibly produced by some other cause. One can state confidently that, in any modern museum of pathology, these bones would be regarded as syphilitic. All of the roentgenologists to whom I have shown the original plate after bringing it to the United States have agreed on the diagnosis of syphilis.

**Other Material:** A large amount of material from Paracas remains to be examined. It is a most promising field for future study.

In November, 1929, I saw a mummy (no. 262) unwrapped by Tello that proved to be of much interest. The body was immediately surrounded by many yards of cotton cloth and finally some cloths embroidered in colors, among which were a few flat, crude gold ornaments and other cultural objects. The knees were flexed against the body. The arms lay over the abdomen. The anterior abdominal wall was intact. Practically none of the soft structures below the middle of the thorax could be identified. The body had evidently been subjected to heat, even to the point of burning. The bifurcation of the trachea,



A



B



C

Fig. 29.—*A* shows the right femur and the left tibia, case 12-7509, after being sawed; *B*, a section of the femur (magnified about from 6 to 7 times); *C*, the tibia (magnified about from 6 to 7 times). (Original photographs.)

the trachea, the larynx and the esophagus, and the parts above them, were well preserved; all were rather dilated; the aorta was not found.

A pathologic condition of much interest was discovered on the roof of the mouth in the form of an ulcer, 26 mm. long and 24 mm. wide, roughly oval, near the median line (fig. 30). It formed a dark, depressed area, from which the mucous membrane had been removed. There was erosion of the bones of the hard palate, with a small perforation just back of the center of the ulcer. The edges of the ulcer were sharply defined. Dr. Pedro Weiss, of the Loayza Hospital, prepared films and sections on the ground. I also brought tissue from the edge of the ulcer and some of the tarry material on its surface

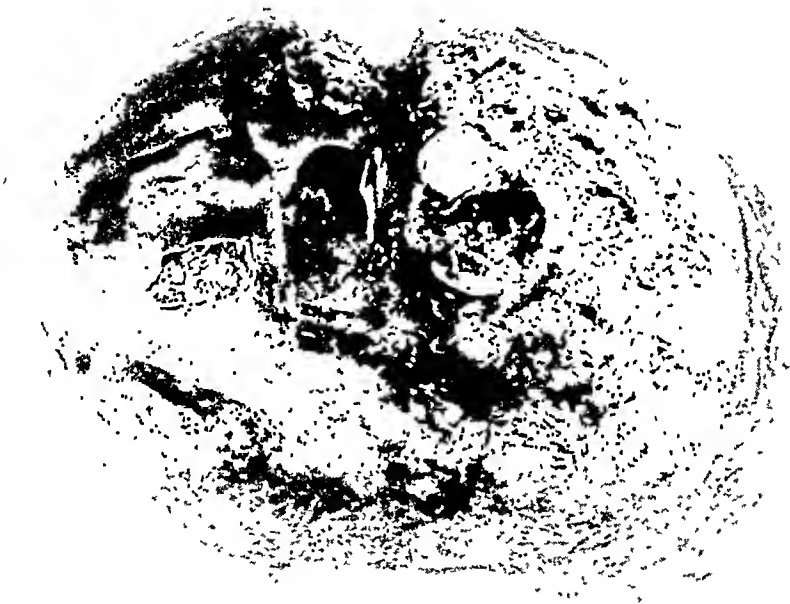


Fig. 30.—Paracas mummy 262. The skull, seen from below. An ulcer of the hard palate is shown. The illustration is enlarged from a small photograph, somewhat retouched (original photograph).

away with me, in the faint hope that spirochetes might be found. However, none were demonstrated either by the Levaditi, Fontana or india ink method; study of the structure with the microscope yielded nothing important. No evidence of syphilis was seen in the nasal or other bones. Some fragments of blood vessels, probably arteries, were obtained from the axilla; no evidence of disease could be detected in them. Nevertheless, I feel certain that the first thought of a physician of the present day, on seeing such an ulcer, would be of syphilis. With fresh material, he could probably make an exact diagnosis.

*Rio Negro Skull from Argentina.*—This specimen was presented at a meeting of the Society of Anthropology of Paris in 1880 by Moreno

(along with another cranium) as an example of a very ancient skull. It had lain at a depth of nearly 4 meters in a layer of sandy, yellowish clay that formed the ancient alluvium of the river that enters the Atlantic Ocean about 6 degrees south and somewhat west of Buenos Aires. It was believed to be from the glacial time of Patagonia, which is more recent than the glacial periods of Europe. The discussion by the members of the society indicated that some of them had doubts of the skull being of so high an antiquity. Hrdlicka (see bibliography in fourth reference), who visited Rio Negro, I believe, about 1910, after examining the locality came to the conclusion that: "In view of the facts presented above, it seems that the two 'fossil' Patagonian skulls have no solid claims to geologic antiquity, the probability being strong that these crania belonged to relatively recent Indian occupants of the region."

I understand that his opinion that the crania were probably those of relatively recent Indians is to be interpreted in a geologic sense, and does not signify that they may not have been pre-Columbian. Hrdlicka said that he did not see the crania in question.

Lehmann-Nitsche said that the pre-Columbian origin of the skull is unquestionable. The specimen is in the museum of the National University at La Plata, Argentina, catalogue number 781. (Lehmann-Nitsche, the well known anthropologist, now of Berlin, was for many years director of the division of anthropology in this museum.) I regret that I have not been able to secure an original photograph of the skull. The illustrations published in the articles mentioned hereafter are not clear enough to permit me to form an opinion.

At the meeting in Paris referred to, the skull was pronounced to be syphilitic by Bordier, Bertillon (Senior) and Broca.

Stegmann wrote a report on the specimens showing disease of bone in the museum at La Plata, in which an account of this skull was included, with a poor illustration. The skull was fragmentary; the face and base and half of the left parietal bone were missing. In certain lesions, beginning above the ridge of the left eyebrow and extending backward, there was evidence of injury, and there might have been traumatic osteomyelitis. The location of the disease process in general, and its appearance, spoke strongly for syphilis. Tuberculosis could be excluded, but on account of the importance of the specimen in medical history, especially that part of it where there was evidence of injury, traumatic osteomyelitis must be considered.

Lehmann-Nitsche brought this skull with him to Germany in 1904, and von Hansemann described it at a meeting of the Berlin Society for Anthropology. He agreed with Stegmann that there had probably been an injury to the left side of the frontal bone, although the lack of any change corresponding to it on the inner surface made that somewhat doubtful. Von Hansemann described the upper surface of the skull as being irregular throughout, with little elevations and depressions that were like scars, except on part of the frontal bone. The scars had smooth edges, well developed near the bregma, diminishing forward, where they no longer appeared to have healed and had rough edges, and perforation had occurred at the



glabella, and nearly so at some other points. The inner surface was for the most part unaffected. No suppurative condition would have produced such smooth scars in bone and such hyperplasia of bone. Tuberculosis, tumor, leprosy and actinomycosis were excluded. Von Hansemann made an unqualified diagnosis of syphilis.

*Four Long Bones from One Skeleton, Undoubtedly Pre-Columbian, from Cañete Valley,<sup>11</sup> Peru.*—The specimens were loaned to me for study by the Field Museum of Natural History, Chicago (catalogue no. 169664), through the kindness of Dr. Berthold Laufer, curator of anthropology. The excavations were made by Dr. A. E. Kroeber, of the University of California, in 1925 (Capt. Marshall Field expedition; see Kroeber, 1926). Dr. Laufer said:

Number 169664 was obtained from Tomb 2, Site A, at Cerro del Oro, Cañete Valley. It was found associated with no pottery or other objects that might assign it to any period; however, the whole site consists of sixteen burials which are, without exception, of the Proto-Nasca type. It is impossible to fix an actual date, but this period dated from somewhere between A.D. 500 and A.D. 1000, probably nearer A.D. 500 than A.D. 1000.<sup>12</sup>

Neither the roentgenograms nor the sawed bones nor the microscopic sections give the pictures to be expected in Paget's osteitis deformans. One is evidently dealing with a chronic inflammatory process, an osteoperiostitis. At certain points, the process is still active, while over large areas it has subsided after having produced much dense, ivory-like bone. That accounts for the unusual weight of these bones. The involvement of four long bones, the distribution of the process over the entire shaft in three, and the absence of sequestrums and of deep sinuses tend to exclude nonsyphilitic periostitis and osteomyelitis. A probable diagnosis of syphilitic osteoperiostitis therefore seems warranted.

The surface of the skull in this case showed no roughening; its sutures were obliterated; it was much flattened anteroposteriorly; such deformation of the skull was common in Peru. The bones involved were the left femur, the two tibias and the left fibula; the other long bones were not remarkable. The involvement of the left fibula was confined to an area about 5 cm. in length at its lower and inner surface, where there were moderate roughening and thickening, evidently periosteal and corresponding to an area of marked roughening and thickening on the left tibia. No illustration of the fibula is given, on account of the slight amount of involvement. The photographs reproduced here show the external surfaces of the left femur and the two tibias; also each of these bones after it had been sawed lengthwise, with its roentgenogram beside it. Photomicrographs of the sections are also given.

The left femur and the tibias are noticeably heavy. The left femur (fig. 31) weighs 435 Gm.; the right, 275 Gm. A femur from another, but normal, ancient

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11. Opens into the Pacific ocean, about 130 kilometers south of Lima.

12. This information was contained in a letter from Dr. Laufer.

Peruvian skeleton, and that of a somewhat larger person, weighs 383 Gm. The length of the left femur is 39 cm. It is slightly curved forward. The entire surface of the shaft is involved, from the lesser trochanter nearly to the knee joint. The shaft is much roughened behind, evidently from new growth of bone. The front is more uniformly thickened and shows numerous stellate superficial scars.

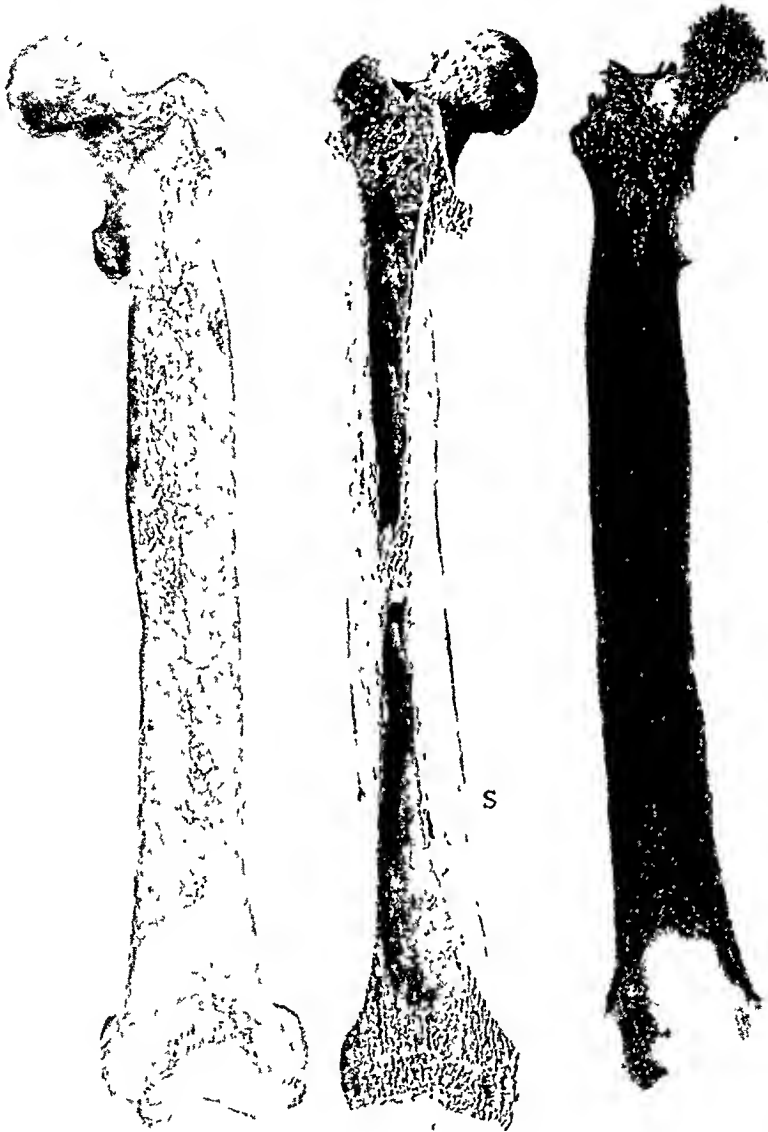


Fig. 31.—The left femur (anterior surface), with the vertical section and the roentgenogram, Field Museum 169664. This femur and the bones shown in figures 32 to 36, as well as some of the photographs and roentgenograms, were loaned to me by the Field Museum, Chicago. All the bones are from a prehistoric skeleton excavated from the Cañete Valley, Peru. *S* indicates the point at which a section was taken for microscopic examination (see fig. 34).

The lower part of the anterior surface looks porous, showing many pointlike perforations. The femur (and the same holds true of the other bones) was sawed longitudinally with great difficulty on account of the dense, ivory-like character of

the tissue. The thickening is most marked along the middle of the shaft. Apparently all layers of the bone participate. There is some encroachment on the medullary canal, but there is still greater new growth of periosteal bone. It is noteworthy that the architecture of the interior of the bone is perfectly preserved, well shown in the cancellous bone at the extremities; but the lamina that separates the lesser trochanter from the rest of the head is unusually thick and is as dense as ivory.



Fig. 32.—The left tibia (anterior surface), the vertical section and the roentgenogram, Field Museum 169664.

The right and left tibia are much alike. Both measure 33 cm. in length; both are heavy. The left (fig. 32) weighs 350 Gm., and the right (fig. 33), 364 Gm. They are decidedly enlarged anteroposteriorly, as is shown where the right tibia was sawed through on that plane, measuring 5.5 cm. at the junction of the upper middle third. In both tibias, the greater part of the outer surface is involved, except that part near the knee joint. There are many osteophytic deposits, evidently periosteal in origin, while the general surface shows large areas punctured with fine openings. In general, the inner surfaces are somewhat more involved than the outer. Both tibias show on their inner surfaces three or four irregularly

defined areas of erosion, which are superficial, extending into the bone to a depth of from 2 to 4 mm. and giving the impression that some process, probably inflammatory, was still actively at work. At other points, the process was quiescent and had terminated with the formation of dense, ivory-like bone. As in the case of the femur, the structure of the cancellous bone at the ends is beautifully preserved, and it has normal architecture. Periosteal new bone formation is plainly shown on



Fig. 33.—The right tibia (anterior and lateral surfaces), the vertical section and the roentgenogram, Field Museum 169664.

the inner side of the left tibia near the point *S* on the sawed bone. In both tibias there is some encroachment on the medullary portion of the bone. The new formation on the outer and posterior part is dense and ivory-like, while the inner and anterior parts are more spongy. The spaces of this spongy bone are large, from 1 to 2 mm. in diameter, and run parallel with the long axis of the bone. No sequestrums and no sinuses leading deep into the bone are seen.

A section of the femur (fig. 34) shows a thin layer of periosteal lamellae parallel with the surface and a projecting osteophyte. Sharpey's fibers can be seen. Haversian systems come close to these lamellae. They show slight irregularity in



Fig. 34.—A section of the femur, Field Museum 169664, after decalcification and hematoxylin-eosin staining;  $\times 12$ . (Original photograph.)



Fig. 35.—A section of the left tibia, Field Museum 169664, after decalcification and hematoxylin-eosin staining;  $\times 12$ . (Original photograph.)

distribution, possibly mosaic structures. I should call this somewhat irregular bone formation from osteitis.

A section of the left tibia (fig. 35) was taken at a point below the entrance of the nutrient artery shown in the illustration of the sawed bone; that included in the photomicrograph is therefore chiefly periosteal in origin. However, it consists of well formed haversian systems chiefly, which are regular in their distribution. They come close to the superficial lamellae, of which there is a thin layer parallel with the surface; at one point, not shown in the illustration, the superficial lamellae are deficient. What appears to be the line of the original periosteal surface of the bone appears at the right of the middle of the photomicrograph (Sharpey's fibers can be seen in torn lamellae at this level).

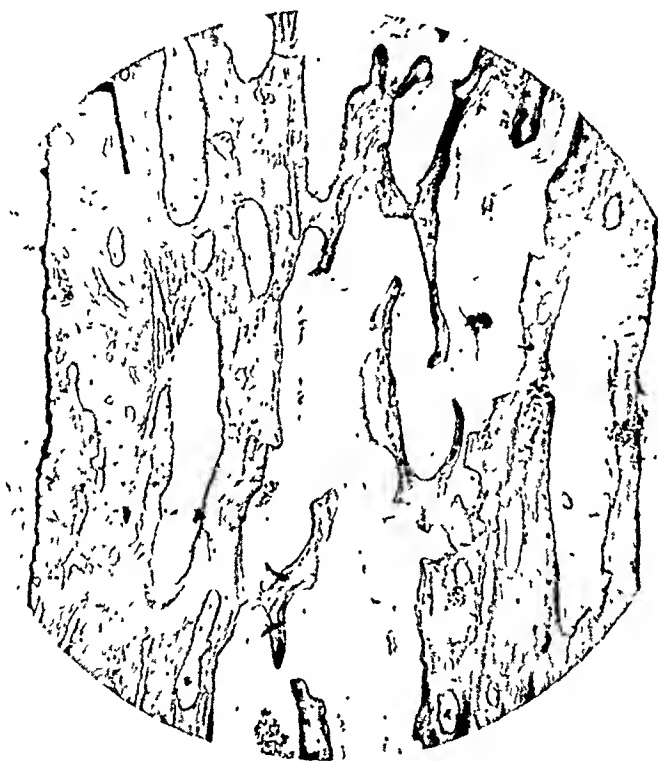


Fig. 36.—A section of the right tibia, Field Museum 169664, after decalcification and hematoxylin-eosin staining;  $\times 12$ . (Original photograph.)

In the right tibia (fig. 36), at points, a few periosteal lamellae running parallel with the surface are seen. They are not continuous. Moderately dense bone with well developed haversian systems comes close to the surface. Below this is rather loose cancellous bone, with its spaces running longitudinally. The general impression given is that of a regular structure.

All of these sections are made from pieces cut in the long axis of the bone so as to preserve the gross appearance as well as possible. The points from which they are taken are indicated on the photographs by the letter S. All were prepared by decalcification, embedding in celloidin and staining with hematoxylin and eosin. No nuclear stain has been secured in any case. Numerous large and small particles from the soil and what are probably spores of molds and other undetermined

foreign materials are found not only on the surface, but in the deepest parts of the sections of bone.

*Long Bones of Mound Builders of Ohio.*—The earliest reference to the possible occurrence of syphilis in ancient Indian bones from Ohio that I have encountered is in the excellent article of Landon, 1881. He described the diseases shown in bones from the prehistoric cemetery at Madisonville, Ohio. He suggested the possibility of syphilis being the cause of some of the lesions encountered, such as hyperostosis, exostoses and evidence of osteitis. Some lesions were bilateral on the tibias. He gave some fair drawings. Landon did not treat of this aspect of his subject at great length, and he took a reasonable position.

Certain diseased bones found at Baum village in Ohio were described by Orton. The implements and other objects from this locality were pronounced by Mills to be pre-Columbian and to belong to the Fort Ancient culture. The bone specimens were studied at the University of Pennsylvania under the direction of Charles H. Frazier and Allen J. Smith. They consisted of eight tibias, one clavicle and an external cuneiform united by bony ankylosis to the third metatarsal; the last was considered to be due to arthritis. The diagnosis of syphilis was made chiefly on the occurrence of the disease in long bones exposed to injury and on the simultaneous occurrence of rarefying and condensing osteitis. Ground sections of six tibias were examined with the microscope. One of these (no. 7) apparently might have been from a case of Paget's osteitis deformans or of some other condition causing rarefaction of bone. The periosteal formation of new bone in several specimens, especially in Orton's no. 9, seemed to be the feature that was the most convincing for syphilis, sinuses and sequestrums being absent. A probable diagnosis of syphilis for some of the specimens was apparently justified, as far as that is possible for single long bones.

Others of the Mound Builder bones from Ohio were described in 1925 by Means, whose conclusions were based exclusively on the gross appearances and the roentgenograms. Means believed that he was the first to use the x-rays for the study of disease in ancient bones. He said: "Definite gross and roentgenological evidence of syphilis was found in three skeletons. They presented varying degrees of involvement from a simple periosteal thickening and roughening on the crest of the tibia, to thickened ivory-like bone invading the medullary canal with gumma formation. . . . The tibias were involved in all three individuals, in one case both, in another one, the more advanced case, a tibia and ulna." He saw several other more or less suspicious specimens.

Means' article is accompanied by excellent reproductions of his roentgenograms. Two of his specimens are among those described in the present article on the pages directly following (cases 1 and 2).

I have seen the clavicle that is shown in Means' plate I, no. 4, and the roentgenograms of it, and I doubt the correctness of the diagnosis of syphilis for this particular bone.

On April 2, 1931, I had the privilege of seeing a large number of ancient Indian bones at the Ohio State Museum, Columbus, Ohio. The director, Dr. H. C. Shetrone, loaned me some of these bones so that they might be examined more closely; all of them were believed by Shetrone to be prehistoric. Specimens were selected from about fifteen individuals, only those bones being taken that offered good promise of being syphilitic. The roentgenograms were submitted to three different roentgenologists, and only those cases were used in which they agreed that the condition was a new growth of bone resulting from periostitis or osteoperiostitis in all probability due to syphilis. In accordance with these tests, nine of the fifteen cases were pronounced to be, in all probability, syphilis. Seven of the nine cases were from prehistoric Mound Builders (four of the Fort Ancient culture, two from the Gartner site and one from burial no. 4, mound 2, Hopewell). The remaining two of the nine cases were from prehistoric Iroquoian sites in northern Ohio (Tuttle site and Taylor site, each one).

It is certain that the bones that I have seen are only a portion of the ancient diseased, probably syphilitic, bones that have been disclosed by the investigations of the Ohio State Museum, but it is probable that they represent the most striking specimens found in recent years. It is impossible in this article to reproduce photographs, roentgenograms and sections from all the nine cases referred to. Six tibias from four of the cases have been selected as illustrative of the whole group. After they had been roentgenographed, they were sawed vertically and small bits were taken for examination with the microscope. Three of the tibias are apparently the same as three of those described by Means.

We have, then, the authority of one of the most modern and the most experienced investigators of Mound Builder remains (Shetrone) for the statement that the bones just described are prehistoric. The gross appearance of these bones, especially after they have been sawed, shows new periosteal growth of bone. In some of them, the new growth of bone encroaches on the medullary canal. This is confirmed by the examination with the roentgenogram and the microscope. The condition seems not to have been the result of Paget's osteitis deformans, but to have been due to periostitis. The absence of sinuses and sequestrums is against a periosteal growth in connection with infectious osteomyelitis. The extent of the process in all of these bones is against periostitis due to simple traumatism. The results favor a diagnosis of syphilis as far as that is possible in the case of dried long bones without other evidence. The occurrence of lesions in both tibias in two of the cases is also in favor of a diagnosis of syphilis.



CASE 1.—Right Tibia, Fort Ancient Culture, Ohio State Museum No. 52 (Figs. 37 and 38): This seems to me to be one of the specimens described by Means (his number 1, plate 1). The length is 39 cm.; the weight, 240 Gm. The area of involvement includes about one half of the length of the shaft, coming somewhat nearer the upper end than the lower end. The enlargement over this area is

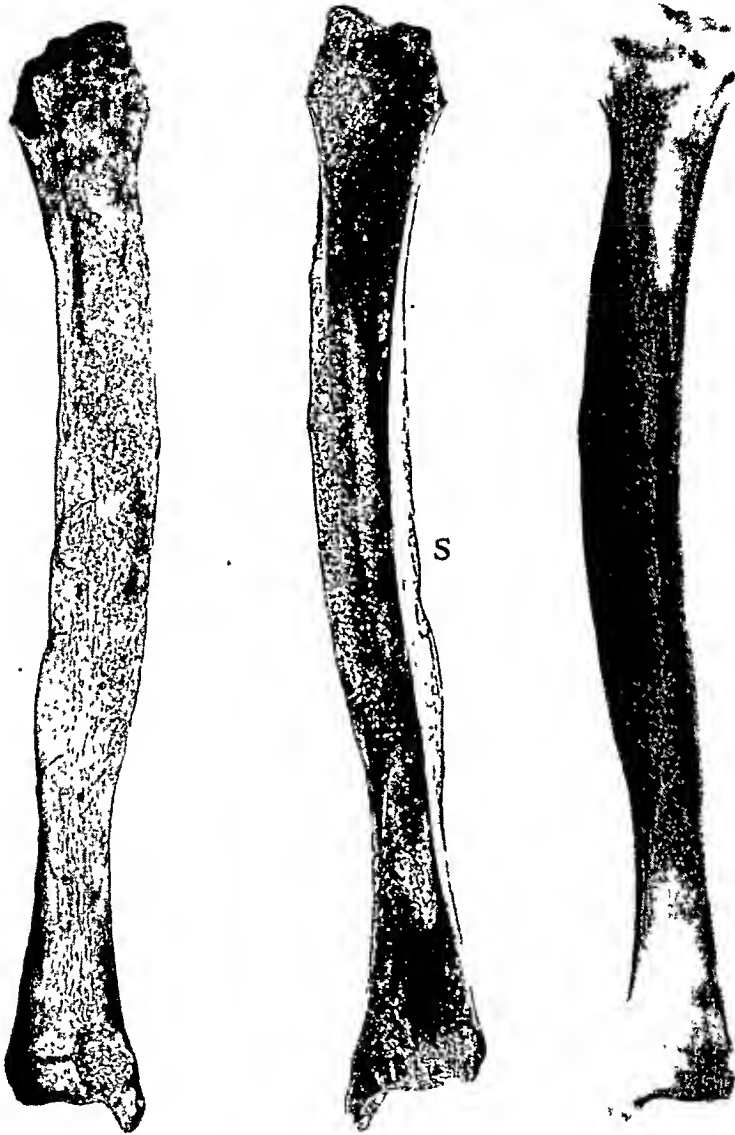


Fig. 37.—Long bone of a prehistoric Mound Builder, Ohio, Fort Ancient Culture. The right tibia (anterior surface), the vertical section and the roentgenogram (by Dr. E. C. König, Buffalo General Hospital) are shown. (Case 1 from Ohio, this article.) (Original photographs.)

notable; it comes to an end abruptly at about the beginning of the lower third. Near the upper end is a small group of osteophytes (not very well shown in the photograph). In general, the surface of the area of involvement is rather smooth, except that it is perforated by many small openings for vessels, most of them run-

ning longitudinally. After sawing, the section of the bones shows a dense, ivory-like thickening, evidently periosteal in origin, along the outer border. The inner border is more thickened than the outer (1.5 cm.), consisting of mostly cancellous bone encroaching slightly on the medullary canal. Through the center of this area of thickening runs a lamina of denser bone (not shown well in the photograph) that appears to mark the original outline of the shaft, so that about one half of this new growth is periosteal in origin. The periosteal nature of the new growth is shown in the roentgenogram. The architecture of the medullary portion of the bone near the extremities is well preserved and perfectly normal.

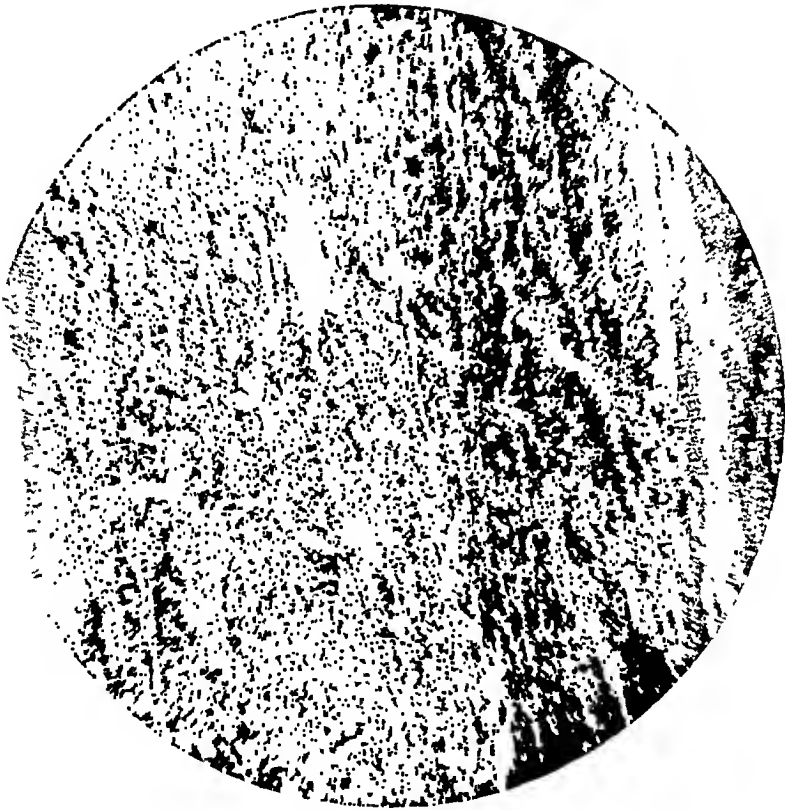


Fig. 38.—A ground section of the tibia ( $\times 12$ ) in case 1 from Ohio, this article. (Original photograph.)

CASE 2.—Right and Left Tibias, Fort Ancient Culture, Ohio State Museum No. 46 (Figs. 39 and 40): This seems to be one of the cases described by Means (his number 2, plate 1). The bones are badly broken. They feel light and are friable, as though most of the organic matter had been lost. The photographs show that one of them cracked badly in the process of sawing. Both of these tibias present thickening on the anterior aspect; the inner and outer surfaces are involved in each case. The thickening, which is slightly nodular, involves the upper two thirds of the shaft, coming close to the head. The surface is, in the main, smooth and rounded, but is beset with innumerable well marked grooves running in the long axes of these bones; the grooves presumably carried blood vessels; in some places there are fine, punctate openings. In the sawed bones, the periosteal nature of the

thickening is evident; this also appears distinctly in the roentgenograms. The medullary canal is large. The architecture of the cancellous bone at the extremities, as far as it has been preserved, appears normal.

CASE 3.—Left Tibia, Fort Ancient Culture, Ohio State Museum No. 78 (Fig. 41): The length of the bone is 41 cm.; the weight, 254.5 Gm. Slightly more than the lower two thirds of the bone is involved along the anterior and inner surface. Thickening of the bone becomes notable about the junction of the lower and middle third; the photograph fails to represent this thickening adequately. Near the middle of the region of the thickening, over an oval-shaped area about

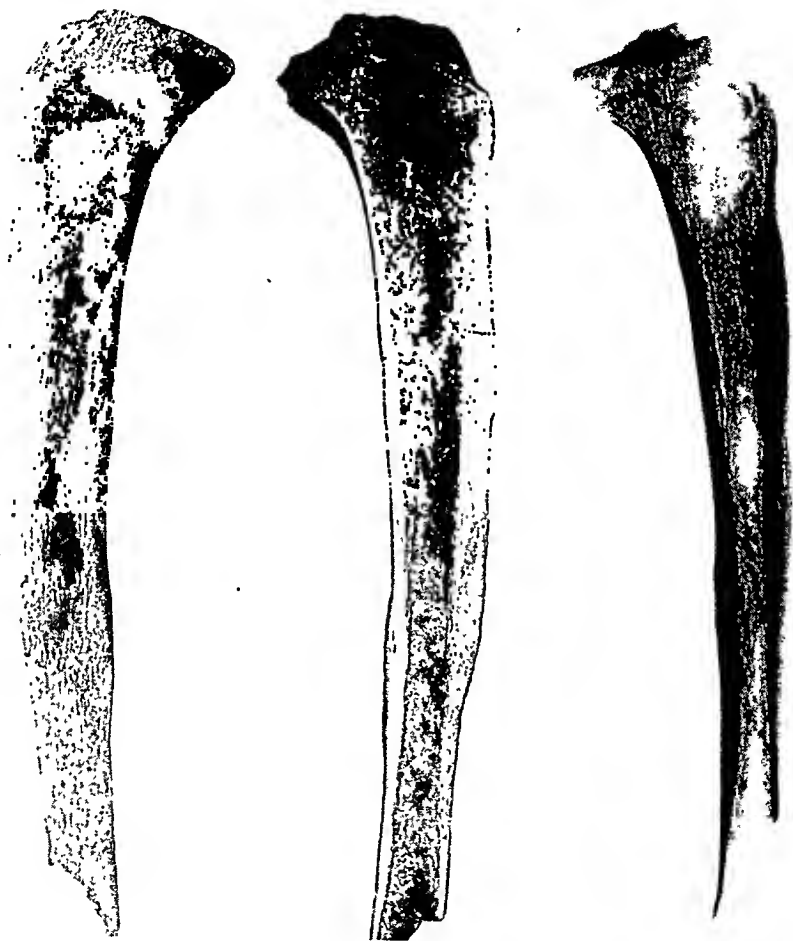


Fig. 39.—Long bone of a prehistoric Mound Builder, Ohio, Fort Ancient Culture. The right tibia (anterior surface), the vertical section and the roentgenogram are shown. The left tibia in the same case is shown in figure 40. This is case 2 from Ohio, this article. (Original photographs.)

4 cm. in length, the surface is perforated with a multitude of minute openings (this could represent an active gumma). The surface of the shaft over a considerable area above the malleolus is very irregular from the formation of osteophytic growths with numerous small perforations; there is some formation of osteophytes along the posterior and inner border in the upper part of the shaft. In general, the upper part of the area of involvement is smooth, except for fine lines running longitudinally. The roentgenogram and the longitudinal section of the bone show

thickening along the anterior border. This thickening apparently is chiefly from periosteal growth with some participation also of the osseous tissue below. The new growth of bone is fairly dense, but that toward the interior of the shaft is in part cancellous. The medullary part of the bone toward the ends displays a perfectly normal architecture and is well preserved.

CASE 4.—Right and Left Tibias, Burial No. 4, Mound 2, Hopewell, Ohio, State Museum, No. 589 (Figs. 42, 43 and 44): Both tibias are 36 cm. in length; the left weighs 156 Gm., and the right, 158 Gm. Both of these tibias present along the



Fig. 40 —The left tibia of the same Mound Builder as was the right tibia shown in figure 39 (case 2 from Ohio, this article). The roentgenograms of both tibias were made by Dr. E. C. König, Buffalo General Hospital. (Original photographs.)

anterior borders, about the middle of the shaft, nodular thickenings with smooth and rounded surfaces, on the right 6 cm. and on the left 10 cm. in length. The surface of the bone over these areas shows many punctate or linear depressions, evidently for the transmission of vessels. The roentgenograms and the sections, after the bones had been sawed longitudinally, demonstrate that the thickening is almost wholly periosteal; the architecture of the medullary part of the bone at both ends is normal.

As sections in cases 1, 2, 3 and 4 made by decalcification proved unsatisfactory on account of the small amount of organic matter remaining in the bones, sections were made by grinding, with fairly good results. The points from which they were removed are indicated on the photographs by the letter *S*. All of these were photographed under



Fig. 41.—Long bone of a prehistoric Mound Builder, Ohio, Fort Ancient Culture. The left tibia (anterior surface), the vertical section and the roentgenogram (by Dr. E. C. König, Buffalo General Hospital) are shown. This is case 3 from Ohio, this article. (Original photographs.)

low power magnification; only two of the photographs were reproduced, as they represent the conditions prevailing in all four cases (figs. 38 and 44). All sections were made in longitudinal direction. The minute

structure was badly preserved. The lacunae for the bone cells could not be identified. In general, it could be said that the new growth of bone consisted of laminae running parallel with the surface. The line marking the junction of the new growth of periosteal bone with the underlying bone of the shaft was usually plainly seen, though not more so than

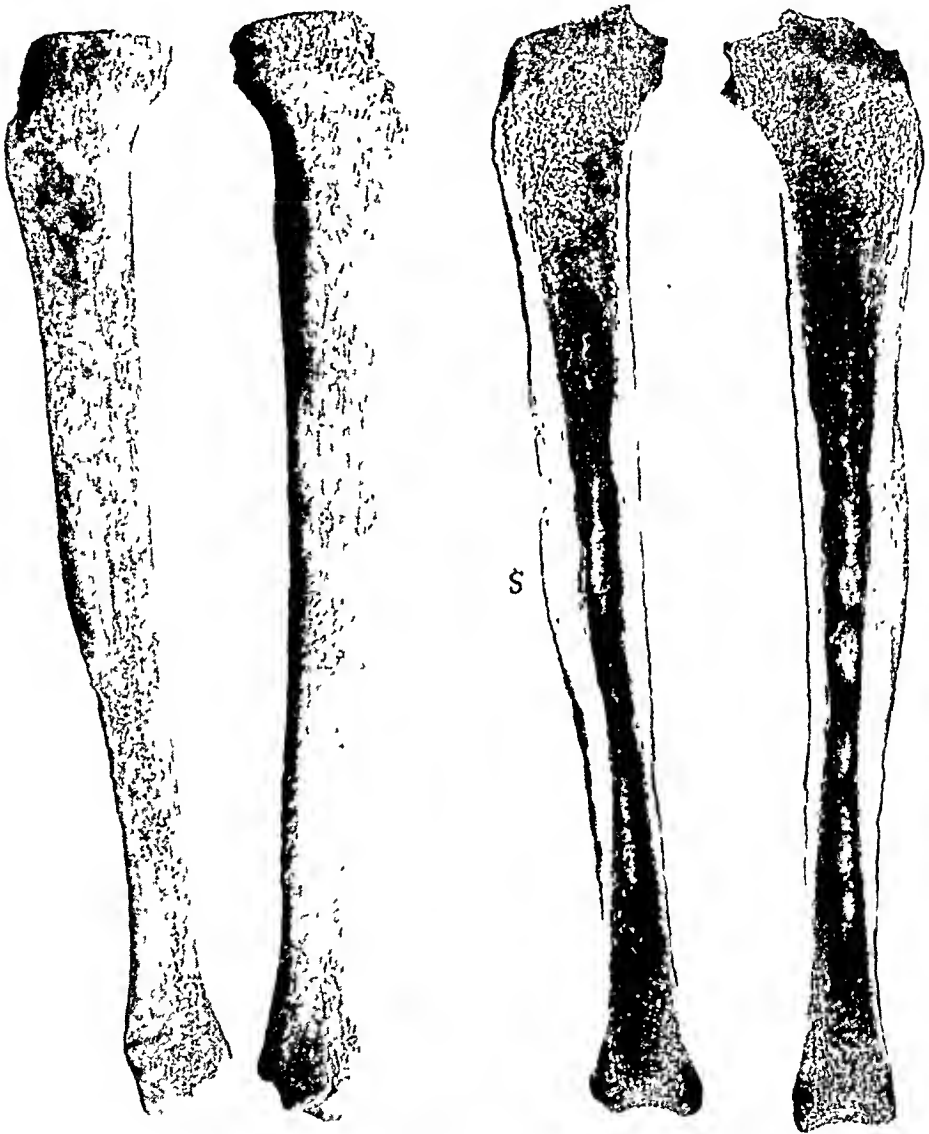


Fig. 42.—Long bones of a prehistoric Mound Builder, Ohio, Hopewell mound no. 2. The right and left tibiae (anterior surfaces) and the vertical sections are shown. This is case 4 from Ohio, this article. (Original photographs.)

in the gross specimen. In general, the impression given was that the new layers of bone were of rather regular formation. In case 1, the organic framework of the outer layers had apparently been dissolved out and the substance later on infiltrated with mineral salts, doubtless

calcium. These layers were divided into small polygonal areas, traversed by fine fibers, which dissolved in acid, but which we were not able to analyze with the polariscope. The spaces for the blood vessels often contained yellowish masses, apparently derived from blood. No red corpuscles could be recognized. This substance appeared in all of the sections examined. There was only a small amount of foreign material.



Fig. 43.—Roentgenogram of tibiae in case 4 from Ohio. This was made by Dr. E. C. König, Buffalo General Hospital.

*Bones from Stone Graves in Tennessee.*—The first to make a serious attempt to demonstrate the antiquity of syphilis in America was Dr. Joseph Jones, professor of chemistry and clinical medicine in the medical department of the University of Louisiana. Jones published a monograph on his studies of Indian remains obtained from explorations of stone graves and mounds in Tennessee, chiefly from the point of view

of an archeologist. The amount of skeletal material that he excavated was apparently not large. Nevertheless, he encountered several skeletons that seemed to him to show evidences of having been affected extensively by syphilis, the exact number of such skeletons or of the individual bones involved not being clear from his text. The number of diseased bones for his total skeletal material was surprising.

No illustrations of the bones in question were given. From the descriptions it appears to me that some, if not all, of these bones were syphilitic. As to the age of the remains, Jones said that they could not be less than from one hundred and seventy-five to two hundred years



Fig. 44.—A ground section of the right tibia ( $\times 12$ ) in case 4 from Ohio. (Original photograph.)

old (in 1876), and that they might be much older (Jones' article, pp. 50, 72 and 175). No implement of European manufacture was found associated with them, he stated, and no metal, except copper, which was, of course, used by the Indians long before the arrival of Europeans. It is possible, however, that the graves and mounds in question were made after the whites came on the ground. Considering that Jones' article appeared more than fifty years ago, his work impresses me as having been well done.

Bloch collected comments on this material. Virchow remarked that the description of the bones did not indicate that "they must be syph-



ilitic." Putnam (an archeologist, not a pathologist), who examined the specimens, said that the disease affecting these bones might be something other than syphilis; he does not say that they were not prehistoric. Klebs, who also saw these bones in 1896, said that "they showed unmistakable syphilitic bone disease." Jones' descriptions of the bones alleged to be syphilitic are given in the excerpt from his article which follows (see also article by Brühl, listed in the bibliography). The only

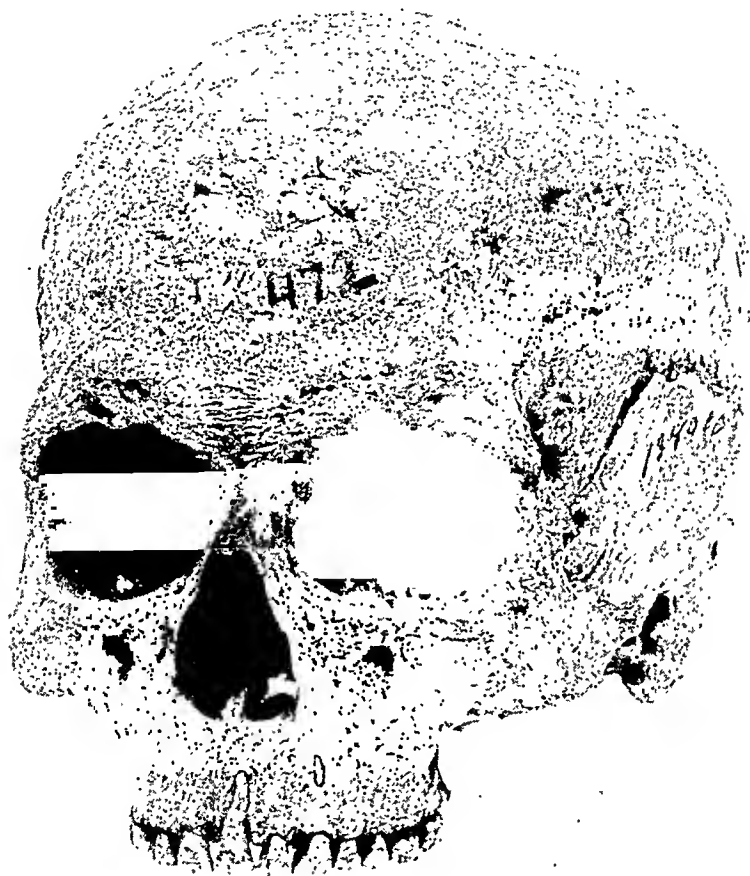


Fig. 45.—A skull from Stone Grave, Big Harpeth River, Tenn., described by Joseph Jones. The photograph was furnished by the Museum of the American Indian, Heye Foundation, New York City, by the courtesy of Dr. Bruno Oettinger.

skull from Jones' collection that I have been able to trace is probably syphilitic, as will be shown in a later paragraph (fig. 45).

Referring to mounds on the Cumberland in Tennessee, Jones (p. 49) stated:

Several of the skeletons in these mounds bore unmistakable marks of the ravages of syphilis. In one skeleton, which appeared to manifest in the greatest degree the ravages of this fearful disease, the bones of the cranium, the long bones

of the arm (the humerus, ulna and radius) and the long bones of the thigh and leg (the femur, tibia and fibula) bore deep erosions, nodes and marks of severe inflammatory action. Many of the long bones were greatly thickened, presenting a nodulated, eroded and enlarged appearance. When sections were made they presented a spongy appearance with an almost complete obliteration of the medullary cavities. The specific gravity of the bones was diminished and the microscopic characters were in all respects similar to those of undoubted cases of constitutional syphilis, which I have observed in my hospital and civil medical practice. Every competent medical observer to whom these bones have been submitted has concurred in the view that syphilis is the only disease which could have produced such profound and universal structural alterations.

Referring to the mounds on the Big Harpeth River, Tennessee, Jones (p. 65) said:

Towards the northern boundary of the mound, in a stone grave immediately at the foot of the two principal graves and at right angles with them, a skeleton was found with the head towards the setting sun. The long bones are strongly marked with syphilitic nodes. The skull is in a good state of preservation, and presents the general conformation of the crania of this ancient race. . . . This cranium had several indentations and nodes on the bones, as if they had been acted upon during life by the syphilitic virus. The external table of the frontal bone appears to have been especially affected. The superciliary ridge is very rough and nodulated, and the nasal bones are thickened, roughened and rounded. The occipital bone shows the effects of pressure which is more marked in the right parietal protuberance, it being much fuller and thrown farther back than the left. The upper extremities of the occipital bone are separated by the transverse suture about one inch in length. (This is possibly the skull that I have seen; Williams, fig. 45.)

I have shown by careful observations that bones taken from stone coffins and burial mounds at Nashville, Franklin, Old Town in Tennessee, and at Hickman in Kentucky, bear unmistakable marks of the ravages of syphilis. The supposition has been advanced that these bones presented merely "traces of periostitis," which were not due to the action of syphilitic poison because "it is uncommon to find shin bones of adults belonging to races clad in skins, and with the lower extremities exposed, in which there is not more or less roughness or hyperostosis along the tibial shafts." So far from these evidences of the action of syphilis being mere traces of periostitis, and constituting mere "roughness or hyperostosis along the tibial shafts," the bones are in many instances thoroughly diseased, enlarged and thickened, with the medullary cavity completely obliterated by the effects of the inflammatory action, and with the surface eroded in many places. These erosions resemble, in all respects, those caused by syphilis, and attended with ulceration of the skin and soft parts during life. Furthermore, the disease was not confined to the "tibial shafts"; the bones of the cranium, the fibula, the ulna, the radius, the clavicle, the sternum, and the bones of the face exhibited unmistakable traces of periostitis, osteitis, endostitis, caries, necrosis, and exostosis. The medullary membrane was evidently involved in many cases to an equal degree with the periosteum; the difference in the appearance of the products of the syphilitic disease being due most probably to the great quantity of fat and other loose tissues, among which the vessels of the medullary membrane run. When thin sections of these bones were carefully examined with the naked eye, and by the aid of magnifying glasses, portions were found resembling cancellous tissue from the enlargement and irregular erosions of the haversian canals, and increase in the number and

size of the lacunae; whilst other portions presented the hardened condition known as sclerosis. I observed in these bones, and especially in those of the cranium, the various forms of osseous ulcerations which have been described by pathologists as characteristic of the action of syphilis, viz., rounded ulcerations with glazed surfaces, and with marked hardening or eburnification of the bone beneath; tuberculated ulcerations, dependent not only on periosteal deposit, but upon chronic inflammation of the compact tissue itself; reticulated ulcerations in which a network of periosteal deposit had been formed, and which had been perforated by ulcers, subsequently forming and assuming the annular type. That these diseases of the bones were not due to mechanical injury, or to exposure to the cold is evident from the fact that they were almost universally symmetrical in their manifestations; thus, when one tibia was diseased the other was similarly affected, both as to the position and nature of the disease. In like manner both fibulae presented similar evidences of periostitis, ostitis and exostosis; this was true also of the bones of the forearm (radius and ulna) and of the clavicle.

The symmetrical distribution of the effects of disease on the two sides of the osseous system could only have resulted from the action of a poison introduced into the blood, and distributed through this medium to all parts of the body.

(Other references similar to these are on Jones' pp. 61 and 85.)

Through Dr. C. W. Duval, of New Orleans, and Dr. S. Bayne-Jones, grandson of Dr. Joseph Jones and professor of bacteriology in Rochester University, it was possible to trace part of the collection of Dr. Joseph Jones to the Museum of the American Indian, Heye Foundation, in New York City. By permission of Dr. Bruno Oettking, curator of physical anthropology in this museum, I was able to examine the skulls in the Jones collection, and among them found one (fig. 45) that perhaps is the specimen described in the foregoing paragraphs (Jones', p. 65); none of the long bones was identified. In my opinion, the case was probably one of syphilis, though not syphilis of the most severe degree.

The skull is large, heavy and well preserved. It shows some deformation and has the cranial capacity stated by Jones. There are fourteen teeth in each of the jaws, all in good condition, not notched, evidently those of an adult. The coronal suture is nearly obliterated. The most marked lesions are in the lower half of the frontal bone, on both sides of the middle line, where the surface is uneven and porotic, and shows about five stellate scars with depressed centers and elevated edges. There are also two or more scars of the same character on the left side of the frontal bone, all plainly shown in the photograph. It did not impress me that there were changes in the nasal bones.

*Other Specimens from America.*—Tennessee: Putnam described a mound in Tennessee certainly more than one hundred and fifty years old (in 1877) where there were two hundred and fifty burials from which he obtained parts of fifty-four skeletons. He said: "Several bones collected in this mound show the effects of disease of some kind, and are such as would generally be called syphilitic; but several pathologists who have examined them unite in stating that they do not

prove the existence of syphilis as other diseases than syphilis might leave such effects."

I believe that these bones are still in the Peabody Museum, Cambridge, Mass. Apparently they are among those referred to in two articles of Whitney, who took a conservative position.

Florida: Certain bones, now in the Army Medical Museum, Washington, D. C., excavated by Clarence B. Moore, of Philadelphia, were the subject of an article by Lamb. According to Lamb, the results of Mr. Moore's work had been published by the Academy of Natural Sciences, Philadelphia, the specimens under discussion coming from a mound named the "Lighthouse Mound," described in Moore's "Additional Mounds of Duval and Clay Counties," Florida, 1896, pp. 24 and 25. It was situated on the north side of St. Johns river at Fernandina, Nassau County, Florida. Lamb quoted Moore as follows:

Exclusive of loose bits of bone, doubtless from the previous excavation, 74 skeletons, all seemingly in anatomical order, were met with and one deposit of charred and calcined human remains. We are, of course, unable to estimate the number of skeletons thrown out or carried away prior to our visit. The first interment was encountered 10 feet in from the southwestern margin of the base. With very few exceptions no art relics lay with the human remains; and if we except a stone hatchet found with a skeleton 8 feet from the surface, and some beads of shell with another interment, no art relics were associated with burials in the body or on the base of the mound.

In no previous work have we found so great a percentage of pathological specimens as in this mound, and, as has not been the case in other mounds, entire skeletons seemed affected, and not one or possibly two bones belonging to a skeleton. The pathological conditions were so marked and cranial nodes so apparent that, in view of the fact that no objects positively indicating white contact were discovered in the mound, though the utmost care was exercised by a trained corps of assistants, we are compelled to regard the bones with the greatest interest since, evidence of contact with the whites being wanting, we must look upon these bones as pre-Columbian in origin. We may state here that all the bones preserved by us came from the depths in the mound which insured their derivation from original burials. These bones found 8 to 12 feet from the surface, and lying beneath numerous undisturbed layers are unmistakably of as early an origin as any yet described and much more reliable than most.

Concerning the specimens, Lamb said:

The bones were at first temporarily, afterwards permanently, deposited in the United States Army Medical Museum in this city (Washington). They are numbered 11,247 to 11,253, Pathological Series, and 3579 to 3583, Provisional Pathological. They consist of the humeri, right ulna, radii, femora, tibia and fibula. They are of the usual dark color and quite friable; the medullary cavity is filled with dark sand and rootlets. There is some platycnemism and pilasterism, conditions so commonly found in aboriginal bones. They show in some places irregular patches of flat, reticulated, hyperostotic growth, in others a more uniform rounded thickening. The illustration shows the appearance of the left tibia and fibula; the ulcerative stage is well marked. The skull was not sent and its condition is not

now known. In the present state of our knowledge I know of no disease except syphilis in which a series of bones of the same skeleton show the lesions illustrated and described. These bones were exhibited by me in the Pathological Museum at the meeting of the British Medical Association at Montreal, and a brief mention of them was made in the *British Medical Journal*, November 20, 1897, page 1487. Professor Osler's remark on seeing the bones was that "This man had the pox." (Lamb's article is accompanied by a fair drawing.)

In April, 1931, I visited the Army Medical Museum and, through Major Callender and Major Ash, was able to examine the specimens deposited in the museum by Clarence B. Moore. The bones described by Lamb were immediately located under the catalogue numbers given by him, and his drawing and description proved to be in general accurate. Roots and sand were still present in the femurs. Places where the left fibula and left femur were broken showed that the new growth of bone was periosteal in origin. The condition of the bones was poor and did not seem to warrant examination with the x-ray or under the microscope. It was my opinion that they were in all probability syphilitic. It seemed somewhat doubtful that the bones could be regarded as certainly pre-Columbian, although archeologists have had a high regard for Moore's work.

The Army Medical Museum contains a quantity of other bones bearing the name of Clarence B. Moore and called "prehistoric syphilis." Apparently they had not been carefully studied and it would be difficult, if not impossible, to prove their antiquity at this date. They are from localities in the southern states. There are some six skulls, only one of which seems to me very suggestive of syphilis, and more than seventy long bones. Many of the latter belong in sets coming from one skeleton. The bones suspected of being syphilitic show irregular thickenings of the shaft; some have a good deal of erosion. The lesions in a few of these may be from osteomyelitis; some may be traumatic in origin; in general, the lesions give the impression of being largely due to syphilis. In view of the new material constantly coming to light, which archeologists are now better able to date accurately than was possible when Moore was at work, it is doubtful that it is now worth while to study his material. The large number of bones probably syphilitic that he found in one region is noteworthy.

The Army Medical Museum has at least three skulls of Indians that are in all probability syphilitic, no. 7427 from Maryland, no. 11,535 from Kentucky and no. 12,815 from Alaska. None of these has any note on the label referring to its antiquity.<sup>13</sup>

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13. Specimen 9667 is stated to be a "skull of a prehistoric Aleut of Chernoffsky, Alaska, collected by Dr. W. H. Dall, Smithsonian Institution." One can be as sure as is ever possible in the case of a dried specimen that this skull is syphilitic. It is to be regretted that it is not now possible to determine whether or not the skull is prehistoric with equal certainty.

In his report on skeletal remains from Arkansas and Louisiana, Hrdlicka (1909) referred to numerous long bones and a few skulls showing inflammatory changes that might have been due to syphilis.

New Mexico: A cranium was excavated at Mitten Rock by Earl H. Morris for the American Museum of Natural History, which now has the specimen (catalogue no. 99-8518). It was described by Shapiro as an example of prehistoric trephining of very ancient date, pre-Pueblo or post-Basket-Maker. The bones were fragile and badly broken but were beautifully repaired in the museum. The skull was not deformed.

The opening made by the operation of trephining, roughly circular, about 50 mm. wide and 40 mm. long, included most of the lower part of the right half of the frontal bone. On its left border below, it entered the frontal sinus. The edges were clean-cut with no signs of repair. It appeared to me that there was a small fistula at the root of the nose near the middle that probably led into the frontal sinus and that might have been the motive for the operation. The entire vault of the skull was rough from nodules and depressions of moderate thickness and depth. The surface was also finely roughened throughout, apparently from erosion with partial healing; at many points erosion still seemed to be active. There may have been a few perforations, though this was somewhat uncertain on account of the damaged condition of the skull. The cranial bones were thin and fragile.

The case is evidently one of periostitis involving almost the entire upper surface of the cranium. Periostitis in this situation and of this extent would usually be attributed to syphilis, but the skull has not the typically syphilitic appearance. However, I have a recent undoubtedly syphilitic skull, the upper surface of which is like this specimen; but in my case the bones are rather thick. On searching for causes that might have produced nonsyphilitic periostitis over such a large area, it occurred to me that scalping or a severe burn, the Indian perhaps being intoxicated, might leave a large area of ulceration that would be months in healing, if it healed at all, and that would undoubtedly affect the periosteum beneath. However, this may well be an atypical example of syphilis of the cranium.

Arizona: The Hemenway expedition to the Salt River valley, Arizona, 1887, brought back a considerable collection of skeletal material, which was preserved and studied in the Army Medical Museum by Dr. Washington Matthews. In his report, he said:

In several cases the condition suggested the possibility, but by no means demonstrated the certainty, of syphilitic disease. Thus in one there was irregular nodular hypertrophy of the shafts of both tibiae, more especially the right, of the lower part of the right fibula, and of the shafts of both ulnae, while the sternal ends of the first ribs showed exostotic growths. In some cases there was hypertrophy of the tibial shafts without any other evidence of disease. The fragmentary and worn condition of the skulls interfere with the recognition of disease and injury.

Colorado: An excellent paper on pre-Columbian syphilis in America was published in 1891 by Hyde. After reviewing the historical aspects of the subject, he described several bones that might be syphilitic, and

offered some photographs. The author seemed himself to be in doubt that the specimens were surely pre-Columbian, and his descriptions and his illustrations do not indicate that they were surely syphilitic. He concluded that syphilis probably existed in America before the arrival of white men, but that proof from bones that were both ancient and syphilitic was not at hand at the time of the writing of his article.

This paper has an additional interest in that Hyde sent to Prudden two diseased tibias that had been given by archeologists, not named, as being from Colorado, and that antedated the Cave Dwellers (Basket Makers?). Prudden's name has been mentioned by several writers on ancient syphilis in America, but as far as I can learn, the report that he sent Hyde, included in the same article, is his only published utterance on this subject. From Prudden's account of these bones, given with characteristic detail and clearness, it is evident that he saw one of the types of bone lesions that have attracted the attention of many observers and that have been illustrated by various examples in this paper. Prudden gave an excellent drawing of a low power microscopic view of the surface of one tibia, showing that the thickening was due to irregular growth of bone that still had recognizable haversian systems. He concluded that the disease was a rarefying and formative osteitis and periostitis that might have been due to syphilis, but he seems to have inclined to the view that it was due to some other cause.

Hyde contributed an article to Morrow's "System of Genito-Urinary Diseases" (1894) that covers about the same ground as the paper just cited. In the second article, he gave illustrations of a skull, the clavicles, femurs and fibulas of a skeleton from Colorado, furnished by Prof. F. W. Putnam, Peabody Museum, Cambridge, Mass. They seem to be the same as those referred to in his first paper. He gives an excellent very low power photomicrograph of a section of one tibia that shows evident periosteal thickening. The photographs of the gross specimens do not permit one to form an opinion. This plate from Hyde is reproduced in D'Arcy Power and Murphy's "System of Syphilis" (vol. 2, plate 2, London, 1908).

Mexico and Central America: Michäelis gave a brief description of a femur, furnished by Hans Virchow from the collection of the department of anatomy of the University of Berlin, said to be from a Toltec grave, and of a date around 1200-1300 A. D. From the gross appearance of the bone and from the evident periosteal origin of the new-formed bone, he regarded the case as probably syphilitic. His illustrations are not clear, but as far as one can judge from them, his opinion is justified (Michäelis, pp. 52-53, plate XXXII).

Gann, who had had an extensive experience in examining Maya ruins, told of a burial mound opened by him in British Honduras, containing a badly preserved skeleton and certain clay figures. The latter seem to indicate the performance of some operation on the penis, and one of them represents a penis, natural size, having three longitudinal incisions extending the length of the upper surface of the glans.

Of the skeleton, the shaft of the right tibia "instead of being triangular, was rounded in section, the prominent angles at the front and sides being obliterated;

it was slightly bowed, with the convexity anteriorly, and it was a good deal enlarged, especially in its upper two thirds, which were composed chiefly of friable, spongy cancellous tissue which rendered the bone much lighter than it appeared. The surface was exceedingly rough, especially in the upper part of the bone, being covered with a number of small nodular outgrowths between which were small pits or depressions. The bone was not examined microscopically. Of the left tibia only small fragments remained but as far as could be judged from these a change somewhat similar to that undergone by the right bone had also taken place here, though not to such a marked extent."

Gann believed that the image of the penis in pottery was intended to represent disease of the organ in the person whose skeleton had been buried, and who had been an example of pre-Columbian syphilis.

Ricketson mentioned a femur in which the "nubbly appearance" of the shaft closely resembled the lesions of a very virulent disease like syphilis.

Peru: Parrot described three skulls, believed to be ancient, coming from Peru, as showing ancient syphilis. The age of the specimens does not seem to have been proved. From the descriptions it would seem that they probably were from cases of symmetrical osteoporosis of the cranium and not of syphilis.

Ashmead (1894-1895) described briefly a skull from Pachacamac, Peru, said to be in the Bandelier collection of the American Museum of Natural History, New York City (he gave no catalogue number). He stated that the specimen was pronounced undoubtedly pre-Columbian by Saville and by Putnam. He said:

In the right supratemporal region of the skull, on the frontoparietal suture, implicating both bones, there is a mark of disease one and a half inches in diameter. The bone is cancellated, eburnated, with deep corrugations as if eaten by disease; the tissue is almost eaten through. . . . To my mind this is an instance of syphilis in a pre-Columbian skull.

It is not possible to determine from Ashmead's description whether or not his conclusion was justified; he gave no illustration. Also, one may question whether or not a pre-Columbian date could be assigned to the specimen with as much certainty as Ashmead indicated. At the present moment, this skull cannot be located in the collection of the American Museum of Natural History.

In the Bavarian National Museum at Munich, Jäger found no less than twelve skulls from Peru on which he made the diagnosis of syphilis. Six of these were from Pachacamac and four from Ancón (both on the coast not far from Lima); for two, the locality was not stated. Apparently all were regarded as ancient, but no facts in proof of their age were cited. The descriptions of the specimens are not convincing that syphilitic lesions were present in a single one of them. For skull 6, Gaffron Collection, a photograph and roentgenogram are



given, from which I should think that the case was one of symmetrical osteoporosis (*cribra parietalia*). In any event, the work is without value for the purposes of this study, in the absence of accurate data to determine the age of these skulls.

The skeletal material described by Tello in his monograph, "Syphilis in Peru" (1909), is said to be deposited in the Warren Museum, Harvard Medical School, Boston. I have examined the Tello collection and am not convinced that any of these specimens is surely syphilitic.

The skeletal material collected by the Yale Peruvian expedition, 1912, at Machu Picchu on the eastern side of the Andes, to the north of Cuzco, was described by Eaton. The material was said to be, for the most part, probably pre-Columbian, but some of it might be post-Columbian. Eaton described a femur and several tibias that very probably presented, at least in part, syphilitic periostitis. He gave excellent photographs and roentgenograms; some of these were reproduced in Moody's "Paleopathology." The skull of a child was described by Eaton as giving evidence of necrosis that might have been due to syphilis or to some other cause.

The skeletal material collected by the Peruvian expedition of Yale University and the National Geographic Society in 1914 and 1915 was examined by MacCurdy (1923).

The cranium of a child from Paucarcancha showed a considerable area of necrosis, chiefly on the left parietal bone, with one small point of perforation. MacCurdy made a diagnosis of probable syphilis, but as no new formation of bone was mentioned, I should think that was doubtful. Another cranium of a child from Patallacta had a circular area of necrosis on the left parietal bone, 4.2 cm. in diameter, which had advanced through the skull. Here also, a diagnosis of probable syphilis was made, but no new formation of bone was described. The cranium of an adult from Patallacta had a circular area of necrosis, including the right frontal and parietal bones, 4.8 cm. in diameter, which was chiefly in the external table, but which showed a perforation of the skull near the center over an area 8 mm. in diameter. There was a small scar on the left parietal bone. From the photograph (MacCurdy's plate XLI) I should think that this might well have been a case of syphilis. MacCurdy referred to a considerable number of long bones from the same region that might well have been examples of syphilitic periostitis. He gave excellent illustrations of all his specimens.

In regard to their antiquity, MacCurdy said: "Whether syphilis existed in the New World prior to the discovery by Columbus cannot be settled by the collection under study here. Some of the burials in the highlands of Peru were evidently post-Columbian." All of the specimens referred to came from points in the Urubamba Valley north of Cuzco.

Hrdlicka, in 1913, collected an enormous number of bones in Peru (see Hrdlicka, third reference). Except for one skull, which was very likely modern, he did not mention any bones showing evidences of

syphilis. Apparently he encountered a considerable number of cases of periostitis and osteoperiostitis in long bones of the coastal region in the north of Peru; he gave no suggestion as to their causation.

Argentina: Other probably syphilitic bones have come from Argentina. Their age cannot be determined so definitely as that of the skull from Rio Negro, described on a previous page. They come from the valley of the Chubut River, which enters the Atlantic around  $8\frac{1}{2}$  degrees south and somewhat west of Buenos Aires. Lehmann-Nitsche said that the excavations were made by an untrained person, before his own arrival in South America. A large number of graves were opened, and a quantity of skeletal material was obtained; the cultural objects were identical with those of native sources, with nothing of European origin. The culture was the same as that described by Verneau in "les Anciens Patagons" from the same territory, which no one doubts was pre-Columbian.

A skull from the forementioned material, which I understand is in the Museum at La Plata, Argentina, was reported in the article of Stegmann.

His description of a surface eaten or gnawed, in some spots depressed and in others having elevations and deposits, ridges, warts and so on, seems to indicate that the skull was typically syphilitic. The disease involved the right frontal and parietal bones, passing on to the left side of the frontal bone and to the parietal bone to a smaller extent. The inner surface apparently showed erosions but no new deposits; the diploe appeared to be involved also, as though by a gummatous process. Stegmann seemed clearly to be inclined to a diagnosis of syphilis, but regarded infectious osteomyelitis as not entirely excluded. The illustrations that he gave appear like those of a typically syphilitic skull. I have not been able to procure an original photograph of this specimen.

Stegmann also described two tibias from the valley of the Chubut, of which he gives illustrations. He made a diagnosis of multiple gummatous osteomyelitis. He described nodes, thickenings and fistulas leading to the inner part of the bone. One of these tibias was afterward submitted to von Hanseemann, who positively rejected the diagnosis of syphilis and regarded the case as one of osteomyelitis or one of tuberculosis.

Hrdlicka (bibliography, fourth reference) also referred briefly to long bones from Argentina that were probably syphilitic, but of uncertain age.

A skull from Calchaqui, Argentina,<sup>14</sup> was described by von Hanseemann (1911). It was thought to be pre-Columbian, but exact data were

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14. It was in the Museum für Völkerkunde, Berlin, catalogue no. I C 8877, La Valeta collection, 1906, in the American division of the museum. Prof. Ludwig Pick and Prof. Eugen Fischer endeavored to secure an original photograph of the specimen for me. Apparently von Hanseemann had not returned the skull at the time of his death, as it was not in the La Valeta collection; nor could it be found in the museum of the Charité.

wanting; the material from Calchaqui was almost entirely free from European influence.

This skull presented on the forehead a great mass of bony scars, mostly with smooth edges, with flat bulging thickenings of the adjacent areas. Over the orbit on the right was a fresh ulceration of bone. There had been also involvement of the nasal region, with healing. Von Hansemann thought the nasal lesions were more like those of lupus; for the skull as a whole, he made an unqualified diagnosis of syphilis. He gave a fair illustration.

Other reports on skulls from South America alleged to be syphilitic that may be included for the sake of completeness are those of Thulie and Vargara. I have examined their articles, and they seem to me not important.

Bloch gave a quite complete review of the evidence for pre-Columbian syphilis to be had in American bones up to the time that his well known work was published.<sup>14a</sup>

#### GENERAL SUMMARY

The diagnosis of syphilis from bones can be made to a practical certainty from a perfectly typical syphilitic skull, such as those described in this paper. Undoubtedly, many skulls that are syphilitic are not perfectly typical and would thus be rejected. The diagnosis from long bones is less certain, but in favorable cases a high degree of probability may be attained. In my experience, the inspection of such bones, especially after they have been sawed longitudinally, gives most of the information that can be obtained; but the x-ray picture may give valuable assistance, and the examination of sections with the microscope is sometimes useful. Several other conditions may produce changes more or less resembling those seen in syphilis of long bones. It is possible, though not probable, that some disease producing changes in the bones like those seen in syphilis may have existed in the remote past and have disappeared in recent times.

#### EASTERN HEMISPHERE

Of a considerable number of alleged finds of ancient syphilitic bones, the following instances are the only ones that I can learn of that are entitled to be called suspicious. It will be observed that they do not concern skulls, but only long bones.

Japan.—A tibia and a fibula described by Adachi and again by Dohi. As far as I can learn, the study of these bones is incomplete, the high antiquity alleged for them is not above suspicion, and no positive diagnosis is warranted.

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14a. Three fourths of the references to American material that were known at the time the book was published (1911) are included.

*Egypt.*—A femur and a tibia alleged to be ancient Nubian, described by Michäelis, who made a diagnosis of probable syphilis, depending on the gross appearance and the examination of sections through the microscope. Nonsyphilitic periostitis could not be entirely excluded; no details are given to prove the antiquity alleged for these bones.

*France.*—There have been so many alleged finds of syphilitic bones in France that, if they were authentic, the old name, morbus gallicus, would seem justified. After careful analysis, three lots emerge which are suspected of being syphilitic:

1. The tibia from Solutré, which was incompletely studied, and which came from a locality rather notorious for having led to mistakes by archeologists in dating their finds.

2. The humerus and ulna from the valley of the Marne, of the de Baye collection. These were first described by Raymond and have been discussed by many others, and diverse opinions have been offered. Sections were made recently by Michäelis, who diagnosed the condition as syphilis on the strength of the gross and microscopic characters; he stated that his photographs and preparations were submitted to Schmorl, who concurred in the diagnosis. A positive conclusion as to these specimens evidently cannot be reached. De Bayes's collections were made between 1874 and 1888. So long a time has elapsed that it might be well to have the antiquity of these bones checked up by one experienced in modern archeologic technic; in such an important case, the possibility of an intrusive burial should be considered.

3. An ulna and a femur with a probable diagnosis of syphilis and a fragment of a femur with an unqualified diagnosis of syphilis, made by Michäelis on the strength of the gross and microscopic characters. These specimens came from the museum at Saint-Germain. They are called neolithic with no other statement as to their origin.

On the negative side, there is a large amount of evidence from the eastern hemisphere. Practically all recent workers in Egypt reported that they failed to find bones showing evidences of ancient syphilis; Elliot Smith's statement that among more than 25,000 skulls examined by him, not one was syphilitic is impressive. The evidence from France was recently reviewed by Jeanselme and by Pales, both of whom decided that it is inconclusive or negative. In 1896, Virchow stated that he did not know of a single ancient syphilitic bone from any locality; Virchow was probably better informed with regard to osseous material collected in Europe than any other person. I cannot learn of any find in any part of Europe outside of France since the time of Virchow (died in 1902) that can be regarded as highly suggestive of syphilis. Bloch stated that he had searched the Hunterian and other collections

in London and Cambridge, with negative results. The late Professor Boldt of Amsterdam, in a personal conversation, informed me that he had studied many thousands of skulls taken from burial places of various periods; he had never found a syphilitic skull of pre-Columbian date; he had in his immense collection several typical syphilitic skulls of later dates. The late Professor Manouvrier of Paris gave me a practically identical report, also in a personal conversation. In other museums I have had the same experience. It is evident that physical anthropologists who handle vast numbers of ancient bones are keenly interested in the problem of syphilis. It is most unlikely that any authentic specimen of pre-Columbian syphilitic skull has been found in Europe and overlooked. Of course there are enormous territories in Asia and Africa that have never been explored in an archeologic sense.

#### AMERICA

The six most important finds will be mentioned first:

1. Specimens from Pecos, N. M.: Case 60455, found by Kidder and described by Hooton, is the most convincing. One has the authority of two of the archeologists and anthropologists of the highest standing, acquainted with all that is best in modern technic, for saying that the specimen is pre-Columbian. This skull is syphilitic as far as it is humanly possible to make a diagnosis on a dried skull. The involvement of a femur from the same skeleton is important. Of two other specimens described from Pecos, one is probably syphilitic, and the other possibly so.

2. Second in importance are the skull and long bones from Paracas, Peru, discovered by Tello. The diagnosis of syphilis is as certain as is ever possible in the case of a dried specimen. I examined the ground at Paracas and concluded that only Tello, who was in charge of the excavations, was in a position to form an opinion as to the antiquity of the specimens. Tello is the best known archeologist in Peru and has had a wide experience in archeologic work in various parts of that country. He is of the opinion that the skull is ancient. Although the possibility of intrusive burial is admitted, it is considered most unlikely.

3. The skull from Rio Negro, Argentina, was said by Lehmann-Nitsche to be undoubtedly prehistoric and was pronounced syphilitic by Broca and other French authorities, also by von Hansemann and probably so by Stegmann.

4. Four long bones from a grave in the Cañete Valley, Peru, pronounced prehistoric (proto-Nasca) by a most competent archeologist (A. V. Kroeber), show marked changes due to an osteoperiostitis, probably syphilitic.

5. Fifth are the Mound Builder bones from Ohio. Four lots of long bones pronounced prehistoric by the latest authority on the Mound Builders (Shetrone) have been carefully studied in this paper. All of these show changes consistent with those produced by syphilis; the new growth of bone is evidently periosteal. The results favor a diagnosis of syphilis.

6. Dr. Joseph Jones of New Orleans was the first to claim that evidence of the occurrence of syphilis in ancient bones exists in America. It seems to me that Dr. Joseph Jones is entirely vindicated. Nowhere in his article did he say that the bones were surely pre-Columbian. He did say that some of them were surely syphilitic. One may be confident that Jones knew syphilis of bone when he saw it, practicing, as he did, about 1876 in New Orleans, with its large colored population. His specimens were seen by Klebs in 1896 and pronounced by the latter undoubtedly syphilitic. I was able to locate one skull from this collection. In my opinion it was, in all reasonable probability, a syphilitic skull, though the process on the skull was of moderate severity.

The aforementioned cases have been selected because they are as nearly free from suspicion as any that can be found. It is proper to repeat in this summary the fact that many long bones of Indians showing evidence of disease resembling syphilis have been found in numerous places both in North and in South America. In contrast with the small number of bones from the eastern hemisphere that are suspected of showing ancient syphilis, the amount of material in America is almost embarrassing. In illustration of this fact, I can relate the following experience. In the spring of 1928, Dr. A. Hrdlicka, of the United States National Museum, loaned me thirteen specimens of long bones showing evidence of disease that might be syphilis. They came from Florida, Louisiana, Arkansas and New Mexico, in the United States, and from Pachacamac and Chicama, Peru. Dr. E. C. König, of the Buffalo General Hospital, made roentgenograms of the lot for me, and assured me that if they had been found as modern bones in the ordinary run of practice, at least ten of the thirteen would have been regarded as showing periostitis, probably syphilitic. Dr. Hrdlicka stated that all of these bones were old and that some of them were in all probability pre-Columbian. He was unable to say, however, that any particular bone was pre-Columbian. None of these bones has been described in this report. They represented only a small part of the bones looking like syphilitic bones in the National Museum. I have already stated that the Army Medical Museum has from various southern states about seventy long bones the condition of which was called pre-Columbian syphilis by Moore. Similar material could undoubtedly be found in

many, if not in most, of the large museums. - The specimens from Ohio described in this report represent only a portion of those collected by Mills and Shetrone. The early articles of Landon on bones from Ohio, Lamb and Moore on bones from Florida, Joseph Jones and Putnam on bones from Tennessee, Hyde on bones from Colorado and Matthews on bones from Arizona show that such diseased bones, suspected of showing syphilis, have long been known to be numerous and were of widespread occurrence. The total number of such long bones from the United States must have been well into the hundreds. Reports from Peru indicate that such bones were equally common there both on the coast and in the highlands (Eaton, MacCurdy); Argentina has also yielded a considerable number.

The age of these specimens can rarely be determined with certainty, but some of them are surely pre-Columbian. There are few cases of osteitis fibrosa or of Paget's osteitis deformans; the changes in most of them are evidently due to osteoperiostitis, without sinuses or sequestrums. Whitney suggested, years ago, that the Indian's mode of life might have rendered him especially liable to periostitis from wounds. I greatly doubt that the Indians suffered more injury than modern man with his machinery, railroads, automobiles and the like. I have examined many ancient Indian bones, and am of the impression that evidences of fractures are no more frequent, even less frequent, in them than in subjects in the dissecting room today.

Another fact to be noted is that much the larger part of the specimens reported from America come from certain regions only. First there is a great area in the United States beginning in Ohio and extending through the southern states to Florida. Another smaller area seems to have existed in New Mexico and the adjacent states. Another area is in Peru, and another in Argentina; possibly there is one in Mexico and Central America. This may be due in part to these regions being the ones where intensive exploration has been done.<sup>15</sup>

It seems to me that the evidence from bones points clearly to the conclusion that the Indians were afflicted with syphilis in a number of parts of America before the arrival of white men. If this conclusion is correct, one may soon expect to have additional evidence, preferably in the form of skulls undoubtedly syphilitic and undoubtedly pre-

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15. When there is more knowledge of the development of immunity in syphilis, that will perhaps throw light on the distribution and spread of syphilis. Certainly, many observers believe that syphilis in the white race was more virulent early in the sixteenth century than it is today, even allowing for improvement in diagnosis and treatment. Recent experimental work also indicates that a certain amount of immunity may be produced in animals under experimental conditions (Chesney).

Columbian.<sup>16</sup> Similar proof from the eastern hemisphere may yet appear, but it has not been produced up to this time.

### COMMENT

A few reflections and even speculations on the significance of the facts related in the preceding pages may be permissible. My interest in tracing the origin of syphilis dates from the year 1909, when I prepared a paper that considered infections native to America. In substance, that paper arrived at the conclusion that aboriginal America was singularly free from the great epidemic diseases that are known to have prevailed in the eastern hemisphere.<sup>17</sup> In the twenty-three years that have followed, I have seen no evidence that would modify the main conclusion, although tularemia has been added to the list of infections, and knowledge of tropical leishmaniasis and of several other diseases has been greatly extended. My original conclusion left open for further study the origin of yellow fever and syphilis. The hope that the origin and the antiquity of syphilis might be determined by lesions of the bones seemed not unreasonable. My single excursion into the field of the study of the history of syphilis (1927) led to comments so diverse that one may well despair of finding documentary evidence that will be convincing to everybody.

The origin of the infectious agent of syphilis has been the subject of fantastic speculations from the sixteenth century down to the recent theory of Thugut, not forgetting the llama theory, which had some adherents (Ashmead, 1895). It is therefore with some hesitation that I permit my imagination to take a modest flight.

Proof that men were living in America prior to the last Glacial Period is wanting at present; very likely a few wanderers were here, whose existence would hardly affect the main question. The end of the last Glacial Period may be roughly dated at about 10,000 years ago. Migrants from Asia have crossed Behring Straits at various times since then and have produced the American Indian race. Somewhere and at some time a nonpathogenic spiral organism acquired pathogenic properties and became *Spirochaeta pallida*. It seems likely that this event

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16. Since this paper was written, Dr. Roy L. Moodie has sent me photographs of bones of a Basket Maker from Arizona, around 1800 years old. The skull, one clavicle, one humerus, one femur and one tibia showed the effects of some disease. As far as I can form an opinion from photographs I should think they were syphilitic. Dr. Moodie will give a complete report later on.

17. A parallel exists in the fact pointed out in a recent article by E. D. Merrill (*Am. Anthropol.*, n. s. **33**:379, 1931), who stated that the domesticated plants of the Old and New World were widely spread, each in its own hemisphere, "but none were common to both regions."



took place in America, less than 10,000 years ago.<sup>18</sup> We are accustomed to associate the occurrence of infections with a dense population and large communities, though new infections have not necessarily originated under such conditions exclusively. In America, large settled communities and dense populations became possible when Indian corn, or maize, was developed and cultivated; before that, the Indians were largely wanderers and hunters, subsisting on fish, shell-fish, game, roots, berries and the like. Botanists inform us that Indian corn was probably developed from the grass teosinte (genus *Euchlaena*), native to the highlands of Mexico and Central America. Its cultivation probably began about 3,000 to 4,000 years ago. Indian corn no longer exists in a wild state. It had been under cultivation long enough for the Indians to have several well marked varieties.

An antiquity for syphilis as slight as even 2,000 years would, I think, present no difficulties to a modern bacteriologist accustomed to the development of rough and smooth, nonvirulent and virulent colonies from one pure culture of bacteria, frequently under conditions that he can easily control.<sup>19</sup> The organism of syphilis has, however, become a parasite of the human body so strict that inoculation of animals produces a disease clinically like syphilis of man only when higher apes are used. It can be cultivated outside of the body with so much difficulty that few strains are under cultivation.

The occurrence of syphilis in pre-Columbian times at points as widely separated as Ohio, New Mexico, Peru and possibly Argentina need present no difficulties. There was plenty of commerce among the Indians. Indian corn was cultivated in all of these areas. Where the seeds of corn could be carried, the seeds of syphilis might also be carried. What now takes place in a few days may then have required centuries, but one is dealing with centuries.

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18. The Indians of tropical America have left behind in their pottery evidence that their minds ran to consideration of sex matters. The large museums often have examples of such pottery, kept behind closed doors, that may be seen on application. The Peruvian Archeological Museum at Lima has an extraordinary collection of this kind of pottery from Peru, some of which represents acts of sexual perversion. This fact may or may not have some bearing on the development of venereal disease.

19. The relationship of syphilis and yaws (frambesia) will ultimately have to be considered in this connection. Some one has said that yaws is "stone-age syphilis." It is also possible that yaws was derived from syphilis. When not only extensive tertiary bone lesions, but also aortic aneurysms are reported in cases of yaws, a pathologist may certainly wonder what he really is dealing with.

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## Notes and News

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**University News, Promotions, Resignations, Appointments, etc.**—In the school of medicine of the George Washington University in Washington, D. C., Leland W. Parr has been appointed associate professor of bacteriology; Roscoe R. Spencer, associate professor of hygiene and preventive medicine; John H. Hanks and Elizabeth Verder, assistant professors of bacteriology, and Alden F. Roe, instructor in bacteriology.

Henry E. Sigerist, director of the institute of medical history in the University of Leipzig, has been appointed professor of the history of medicine in the Johns Hopkins University to succeed William H. Welch.

Walter A. Jacobs, chemotherapist, and Karl Landsteiner, pathologist, of the Rockefeller Institute for Medical Research, have been elected members of the National Academy of Sciences.

**Society News.**—At its recent meeting in Philadelphia the American Society for Experimental Pathology elected Payton Rous president, Carl C. Weller vice-president, and C. Phillips Miller, Jr., secretary-treasurer.

The next meeting of the American Association of Pathologists and Bacteriologists will be held in Washington, D. C., May 2 and 3, 1933, in conjunction with the meetings of the Congress of American Physicians and Surgeons. The president of the association is E. T. Bell; the vice-president, O. T. Avery; the treasurer, F. B. Mallory; the secretary, Howard T. Karsner, and the assistant secretary, Robert A. Moore.

The Italian section of the Societa internazionale di microbiologia will hold its fourth congress next November. Among the subjects to be discussed are microbic dissociation, postvaccinal encephalitis and blood groups in relation to physical constitution.

**Recommendations in Regard to Cancer Control in New York State.**—The New York State Health Commission recommends in its report as follows:

1. That the state and local departments of health promote the establishment of more adequate facilities for the diagnosis and treatment of cancer, including hospital care and diagnostic tumor clinics with properly qualified physicians in charge and an adequate follow-up service.

2. That qualifications for pathologists in tumor diagnosis and standards for pathological laboratories be prescribed by the Public Health Council, in a manner similar to the systems in effect for public health laboratories.

3. That the State Department of Health continue and extend its efforts to educate the public regarding cancer and that voluntary associations carry out a more aggressive education campaign in this field.

4. That the state division of cancer control effect a working relationship with the committee of the Medical Society of the State of New York which is sponsoring the postgraduate instruction of physicians to develop greater knowledge and increased interest on the part of the profession in the early diagnosis and treatment of cancer.

5. That sufficient appropriations be made to continue and expand the research work now in progress at the State Institute for the Study of Malignant Diseases.

**Quarterly Bulletin of the Health Organization of the League of Nations.**—The first number is for March, 1932. This periodical will be devoted to work under the auspices of the Health Organization. Forthcoming numbers will contain reports of technical commissions on tuberculosis, venereal diseases, syphilis, medical education, malaria, cancer, etc. The annual subscription is \$2.

# Abstracts from Current Literature

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## Experimental Pathology and Pathologic Physiology

METABOLISM STUDIES IN CHRONIC HYPERPARATHYROIDISM IN MAN. J. L. JOHNSON and R. M. WILDER, *Ann. J. M. Sc.* **182**:800, 1931.

The repeated injection of parathormone has produced, in puppies and young rats, a uniform skeletal abnormality that is characteristic of osteoplastic fibrous osteitis (von Recklinghausen). A lacunar resorption of the trabeculae and cortex of bones resulted, with replacement by fibrous connective tissue and osteoid tissue. Giant cells appeared and cysts were formed. The administration of viosterol with parathormone did not modify significantly the end-results. The data of these experiments are reported elsewhere. The present paper deals with observations of the metabolism of a normal human subject who was treated experimentally, first with parathormone, then with parathormone and viosterol. The characteristic disturbances of calcium and phosphorus metabolism produced by injections of parathormone, as well as certain symptoms, notably pain in the bones and weakness of the muscles, characteristic of fibrous osteitis, were not significantly modified by the simultaneous administration of viosterol. The results confirm the view that the genesis of fibrous osteitis, as this disease is observed spontaneously in man, is in an oversupply of parathyroid hormone, and that this disease bears no relation, etiologically, either to osteomalacia or to rickets.

FROM AUTHORS' SUMMARY.

PHYSIOLOGICAL RESPONSES AND IMMUNE REACTIONS TO EXTRACTS OF CERTAIN INTESTINAL PARASITES. H. E. ESSEX, J. MARKOWITZ and F. C. MANN, *Am. J. Physiol.* **98**:18, 1931.

Studies of the effect on blood pressure of extracts of certain parasitic worms, *Taenia pisiformis* and *Toxascaris canis*, showed a marked depressor action, comparable in some respects to the effects of rattlesnake and bee venom, but without the features of swelling of red cells, hemolysis and diffuse hemorrhage. In the case of *T. pisiformis*, the depressor action was not manifested in dogs heavily infested with the parasite, indicating an apparent ability to develop immunity to the toxic substances.

H. E. EGGERS.

DOES HEMOPHILIC BLOOD CONTAIN AN EXCESS OF AN ANTICOAGULANT? P. S. EVANS, JR., and W. H. HOWELL, *Am. J. Physiol.* **98**:131, 1931.

Studies of the antiprothrombin content of the blood of a hemophiliac, taken on different days, were made by comparisons with that of three normal persons. No indication whatever was found of excess of the anticoagulant agent, and indeed the results tended to show directly the reverse—less rather than more antiprothrombin. The method of Fuchs for the preparation of the antiprothrombin, with some modifications by the writers, is given with some detail.

H. E. EGGERS.

THE RAPIDITY OF INTERCHANGE BETWEEN BLOOD AND LYMPH IN THE DOG. M. E. FIELD and C. K. DRINKER, *Am. J. Physiol.* **98**:378, 1931.

After an induced sterile inflammatory process in the dog, the capillaries were found to become abnormally permeable to blood proteins, with the result of a marked increase of the protein content of the subcutaneous lymph. After venous

obstruction there is an outpouring of very dilute subcutaneous lymph in which red cells are usually present. With reduction of blood protein by plasmapheresis, there is associated a marked depletion of the protein content of the subcutaneous lymph and of that of the thoracic duct. The flow of lymph here is greatly increased, and its passage through the capillaries and tissues into the lymphatics must occur with little change in its composition.

H. E. EGGERS.

THE EFFECTS OF DENERVATION OF THE LIVER. H. LUNDBERG, J. M. McDONALD, F. C. HILL and L. V. HILLYARD, *Am. J. Physiol.* **98**:602, 605 and 610, 1931.

In this series of three related articles, various phases of the effects of denervation of the liver are discussed. This condition was achieved by section of all the structures in the gastrohepatic omentum except the common bile duct, hepatic artery and portal vein. Where there was any doubt as to the section of the branch from the vagus, both vagi were severed in the thorax. With dogs treated in this way, no constant effect was observed on the amount of bile secreted or on the elimination of pigment, on the value of the blood sugar during fasting, on the hyperglycemia produced by epinephrine, dextrose or pituitary extracts, on the hypoglycemia of insulin, nor on the red cell count, the leukocyte count and the coagulation time of the blood. Nor was the action of peptone, in decreasing the number of leukocytes in blood from the liver, affected.

H. E. EGGERS.

UREA TOLERANCE AFTER UNILATERAL NEPHRECTOMY IN RABBITS. H. T. KARSNER and ASSOCIATES, *J. Exper. Med.* **55**:27, 1932.

The method of study has an objective somewhat different from, and lacking the precision of, the ratio of Addis and the urea clearance of Van Slyke. It serves, however, to demonstrate that although for a month after unilateral nephrectomy the remaining kidney shows diminished capacity to hold blood urea within the normal range, nevertheless after four months this function is maintained in essentially the same degree as if both kidneys were present. This does not imply that all activities of the kidney remaining after unilateral nephrectomy are potentially augmented, but the data offered justify the conclusions that as the remaining kidney undergoes enlargement its functional capacity increases, and that the process represents a genuine hypertrophy in the critical sense.

AUTHORS' SUMMARY.

PROTECTION OF ADRENALECTOMIZED ANIMALS AGAINST BACTERIAL INTOXICATION BY ADRENAL CORTICAL EXTRACT. F. A. HARTMAN and W. J. M. SCOTT, *J. Exper. Med.* **55**:63, 1932.

The resistance of adrenalectomized rats to bacterial intoxication has been significantly increased by an extract of the adrenal cortex. This is shown both for acute intoxication with killed *Bacillus typhosus* and for chronic intoxication with killed *Staphylococcus aureus*. During the height of the bacterial intoxication, relatively large amounts of the cortical hormone are apparently required to maintain the animals. It is considered probable that a human pyogenic infection imposes a severe load on the adrenal cortex.

AUTHORS' SUMMARY.

THE PRODUCTION AND REPAIR OF BONE LESIONS IN EXPERIMENTAL HYPERPARATHYROIDISM. H. L. JAFFE, A. BODANSKY and J. E. BLAIR, *J. Exper. Med.* **55**:139, 1932.

Young guinea-pigs are more susceptible than adult guinea-pigs to the effects of single or repeated doses of parathormone, as shown by the bone changes. Several successive daily doses of parathormone, in rapidly increasing amount, result in an accentuation of the effects. In young and adult guinea-pigs a com-

pensation is established during prolonged treatment with parathormone, which enables them to tolerate repeated large doses, and which permits considerable repair of bone lesions produced earlier in the treatment.

AUTHORS' SUMMARY.

EXPERIMENTAL NEPHRITIS IN THE FROG. J. OLIVER, J. Exper. Med. 55:295, 1932.

The apparent similarities in the functional derangements following vascular and parenchymal alterations in the kidney are in fact evidences of a single and identical functional state that may arise from either cause. Functional testing of the kidney cannot, therefore, suffice to determine the condition existing in the kidney. This can be accomplished only by appreciation of the structural alterations present.

AUTHOR'S SUMMARY.

VASODILATATION IN THE LIMBS IN RESPONSE TO WARMING THE BODY, WITH EVIDENCE FOR SYMPATHETIC VASODILATOR NERVES IN MAN. THOMAS LEWIS and GEORGE W. PICKERING, Heart 16:33, 1931.

A simple method of testing the capacity of arteries of the extremities to dilate is to note the reaction in the cool extremity when the body is exposed to a warm atmosphere. In the hand vasodilatation so induced manifests itself first by a rise of temperature and by brightening in the color of the finger tips. The time at which the response begins is influenced by the initial temperature of the extremities, being delayed by coldness. The response is effected entirely through the sympathetic nerves. By studying the reaction in Raynaud's disease and comparing the effects of ulnar anesthesia, evidence has been obtained that the human sympathetic nerves contain vasodilator as well as vasoconstrictor fibers. The warm chamber is valuable clinically in differentiating obstruction arising from spasm and that arising from structural disease of the arteries. In the former, the response is rapid and proceeds to a high level. In the latter, the response is slow and less pronounced. The chamber can also be used in determining that the sympathetic innervation of vessels of the limb has been lost.

AUTHORS' SUMMARY.

THE IRRESPONSIVENESS AND REFRACTORINESS OF THE VESSELS OF THE SKIN. EDWARD J. WAYNE, Heart 16:53, 1931.

By electrophoresis it is possible to introduce histamine diffusely into the skin, in low concentration. The vasoconstrictor effect responsible for Bier's spots is capable of overcoming a slight local vasodilatation so produced. It will not prevail over a more intense local vasodilatation caused by histamine. In a similar way Bier's spots appear on areas of local vasodilatation caused by injuries of moderate intensity. They do not invade the intensely reddened areas produced by stroking in a case of factitious urticaria. A Bier's spot may receive electrophoretically, without extinction, a dose of histamine far larger than is necessary to redden control skin. It is concluded that the vessels of the skin under the influence of either histamine or H substance liberated by injury behave similarly toward Bier's spots, when the two are distributed in a comparable way through the tissue spaces. The minute vessels of the skin actively dilated under the influence of small doses of histamine introduced by electrophoresis can be completely constricted by a sufficiently large dose of epinephrine hydrochloride. Larger doses of histamine cause the minute vessels to become completely irresponsive to epinephrine. The minute vessels of the skin actively dilated under the influence of H substance released by injuries of varying degrees of severity behave in a similar way. The local vasodilatation due to slight injury, such as a stroke or a scratch, can be completely obliterated by a large dose of epinephrine hydrochloride. Severe injuries to the skin such as are produced by freezing with carbon dioxide snow give a degree of local vasodilatation that is highly resistant to epinephrine. The



skin inside an area of intense blanching by epinephrine may be injured without causing the vessels to dilate. The minute vessels of skin treated with ultraviolet rays show relative irresponsiveness to epinephrine when tested at various times after irradiation. Relative refractoriness to histamine has been demonstrated similarly. Skin treated with mustard oil exhibits irresponsiveness to epinephrine and refractoriness to histamine. Irresponsiveness of the lesions to epinephrine could be demonstrated in five of six cases of psoriasis examined, varying in degree in different cases. Five cases of psoriasis examined showed refractoriness of the lesions to histamine to a greater or less extent. Refractoriness to the stimulus of a stroke over the site of the psoriatic lesion was demonstrated in a case of psoriasis showing dermatographia. Slight irresponsiveness to epinephrine and considerable refractoriness to histamine were shown in a case of exfoliative dermatitis. It is concluded that all the observations are consistent with the view that the vascular reactions resulting from injury to the skin are due to a liberated substance, either histamine or some substance closely allied to it.

#### AUTHOR'S SUMMARY.

THE RELATION OF THE LIVER TO THE DISPOSAL OF HEMOGLOBIN. R. MUIR and J. S. YOUNG, *J. Path. & Bact.* **35**:113, 1932.

When hemoglobin in considerable amounts is introduced into the circulation we fail to find any evidence that it is excreted in the bile. In similar circumstances we also fail to find evidence by microchemical methods that the hemoglobin is taken up by the hepatic cells or by the Kupffer cells. This contrasts with the fact that as a result of hemoglobinemia the renal cells give a distinct iron reaction at a very early stage. The spleen does not show the characteristic swelling that occurs as the result of hemolytic poisons. In chronic hemoglobinemia produced by repeated intraperitoneal injections of hemoglobin solution, an increase of the iron in the liver occurs, but it takes place slowly and does not reach the amount met with in the anemia produced by a hemolytic serum. The reason for this difference is not known. The possibility that in such experiments the iron is carried from local deposits to the liver in some form other than hemoglobin cannot be excluded. In chronic hemoglobinemia, the outstanding feature is the large accumulation of granular hemosiderin in the renal cells, the percentage of iron in them being much higher than in the hepatic cells. The experiments, taken as a whole, have not given support to the view that hemoglobin as such is taken up by the cells of the liver and broken up by them.

#### AUTHORS' SUMMARY.

RESEARCHES ON BERIBERI. NOEL BERNARD, *Ann. Inst. Pasteur* **47**:508, 1931.

The etiology of beriberi is exhaustively considered in an article of seventy-two pages. "In résumé, beriberi appears to be a toxic infection, dependent on three essential factors solidly aligned: (1) the toxic organism; (2) a gastrointestinal medium favoring its action; (3) a state of poor organic resistance resulting from the alimentary régime. If one of these three factors fails, although two others may favor the disease, beriberi will not occur." The author considers extensively the gram-positive sporulating aerobe of the *Bacillus megatherium* group, *B. asthenogenes*, which he has been studying for some years.

#### M. S. MARSHALL.

THE EXCRETION OF SOME DRUGS AND DYES IN THE SPUTUM. J. HERMS, *Beitr. z. klin. d. Tuberk.* **78**:613, 1931.

The permeability of the bronchial mucosa is similar to that of the salivary glands and that of the gastric mucosa; that is, cations are electively excreted, while anions with the exception of the halogens are not excreted. In inflammation, the alveolar epithelium is permeable for anions and only irregularly for cations.

The permeability can be tested in man most easily with the anion, salicylic acid, and with the cation, antipyrine. The author points out how alterations in the permeability of the respiratory tract can be utilized for diagnostic purposes.

MAX PINNER.

GASTRIC SECRETION IN PULMONARY TUBERCULOSIS. K. MENZEL and M. K. SCHÖFFEL, *Beitr. z. klin. d. Tuberk.* **78**:654, 1931.

In 100 patients with pulmonary tuberculosis the gastric secretion was studied by the fractionating method. Sixty per cent of the patients showed hyperacidity, 24 per cent normal acidity, 9 per cent hypo-acidity and 7 per cent anacidity. Hyperacidity was particularly frequent in productive disseminating forms and during exacerbations. Only one of the seven patients with anacidity had complete anacidity; that is, this one did not react with acid secretion after histamine, neutral red and insulin had been administered. Symptoms are most marked in patients with hypo-acidity.

MAX PINNER.

ICTERUS DUE TO ARSPHENAMINE. L. STRAUZ, *Dermat. Wchnschr.* **93**:1813, 1931.

Rabbits were poisoned with large doses of neoarsphenamine (from 0.6 to 2.4 Gm. per kilogram). The smallest doses caused passive hyperemia of the liver and small interstitial hemorrhages. Later, fat infiltration in the otherwise well stained cells was noted. More severe injury to the parenchyma was evidenced by cloudy swelling and fatty degeneration, and later the nuclei would not stain. The degeneration extended over a large number of cells. Necrotic foci appeared, which were separated from the undamaged cells by a wall of leukocytes. These infarct-like necroses were due to embolic changes in the arterioles. Masses of bile appeared as a result of the damage to the hepatic cells. The degree of damage to the liver closely paralleled the dosage, but widespread hepatic necrosis could not be caused, even with massive doses. The lesions were focal and apparently the result of embolic processes. In the gallbladder, degenerative changes were observed in the epithelium and varied in intensity with the amount of the drug given. Fat droplets appeared in the cells and, resulting from large doses, rather extensive necrosis was seen. Marked fat deposits were found in the fibrous layer of the wall, similar to those seen in the liver; also focal necroses, possibly infarcts. The author concludes that regressive changes occur in the liver and gallbladder in arsphenamine poisoning. The frequent occurrence of necrotic foci resembling infarcts was striking, although definite vascular changes were not found to account for their presence.

LAWRENCE PARSONS.

### Pathologic Anatomy

DESTRUCTION OF HISTIOCYTES OF THE BLOOD. C. CLASING, *Virchows Arch. f. path. Anat.* **277**:143, 1930.

White mice were given intravenous injections of india ink or of carmine, and were killed at intervals varying from one-half hour to five months. Immediately following the injection, the endothelial cells of the liver, spleen, lungs, bone marrow, suprarenal glands and intestines took up the injected material. After a time these cells became separated from the tissues in which they lay and entered the blood stream to become the histiocytes of the blood. They were removed from the blood in the capillaries of the liver and spleen, where they were destroyed, in which organs the deposition of large amounts of the injected material takes place. Clasing could find no evidence of destruction of the histiocytes within the lungs.

W. SAPHIR.

EXPERIMENTAL HEMATOPORPHYROSIS OF THE BONES. H. HAMMER, Virchows Arch. f. path. Anat. **277**:159, 1930.

Rats, rabbits, guinea-pigs and one dog were given daily injections of artificial hematoporphyrin for periods varying from a few days to several weeks. As in previous experiments with the natural uroporphyrin, there occurred a typical reddish-brown discoloration of the skeleton and the teeth in growing animals. Adult animals remained unaffected unless an experimental fracture of a bone was produced, in which case the callus and newly formed bone assumed a deep red color as the result of deposition of porphyrin. The porphyrin deposited in the bones and teeth disappeared after a variable period, apparently as the result of its decomposition.

W. SAPHIR.

HISTOLOGIC CHANGES FOLLOWING THE PARENTERAL INTRODUCTION OF AUTOGENOUS PROTEIN. W. JELIN, Virchows Arch. f. path. Anat. **277**:221, 1930.

Twenty guinea-pigs were used in the series of experiments reported here. Two cubic centimeters of blood was withdrawn by cardiac puncture from each animal, and after separation of the serum the latter was injected into the peritoneal cavity of the same animal. The animals were killed at intervals varying from twenty-four to one hundred and five hours. The histologic changes noted were similar to those previously described by the author following the injection of heterologous protein. These changes were: hyperplasia of the reticulo-endothelial system of the liver, spleen and lymph nodes; dilatation of the capillaries and larger vessels of all the internal organs, with inflammatory reaction of the walls of the vessel; disturbance of the carbohydrate metabolism of the liver, characterized by marked glycogen infiltration of the liver and hyperglycemia; a slowly developing glomerulonephritis, with congestion, minute hemorrhages and cellular infiltration of the glomeruli and degeneration of the tubular epithelium; enlargement, hyperplasia and increased pigment formation in the suprarenal glands.

W. SAPHIR.

THROMBOSIS OF THE INFERIOR VENA CAVA IN A NEW-BORN INFANT. W. EILERS, Virchows Arch. f. path. Anat. **277**:248, 1930.

Thrombosis of the inferior vena cava occurred in an infant 15 days old. The thrombosis was followed by hemorrhagic infarction of both kidneys and both suprarenal glands. Thrombosis was also noted in the dural sinuses. Since inflammation was present in the region of the umbilicus, the thrombosis is considered to have been infectious in origin.

W. SAPHIR.

PERIRENAL HEMORRHAGE. P. HEILMANN, Virchows Arch. f. path. Anat. **277**:256, 1930.

Four cases are described in which large perirenal hematomas were found. In two, the condition was discovered in the course of a surgical operation, and in two, at necropsy. Of the latter, one was a case of scarlet fever; the other, a case of nephrosclerosis with cerebral hemorrhage. The hemorrhage was unilateral in each case, and no apparent cause for it could be found. The possibility of reflex nervous action is discussed. Histologic examination revealed necrosis of the capillary endothelium and pericapillary leukocytic infiltration.

W. SAPHIR.

CHANGES IN THE HEAD OF THE FEMUR FOLLOWING FRACTURE OF THE NECK. E. Freund, Virchows Arch. f. path. Anat. **277**:326, 1930.

The changes that occur in the head of the femur following fracture of the neck of the femur depend on the degree to which the blood supply of the head is involved. If the blood supply is completely abolished, the bone and the marrow become necrotic and may undergo partial resorption. The cartilage of the head is

usually preserved, because it obtains adequate nutrition from the synovial fluid. The extent to which the blood supply of the head is interfered with by fracture of the neck depends on the degree of injury to the round ligament. If the blood vessels of the ligament remain intact, partial necrosis of the head may be followed by new formation of bone and restitution of the head.

W. SAPHIR.

**DIVERTICULUM OF THE HEART.** S. MAHRBURG, *Virchows Arch. f. path. Anat.* **277**:498, 1930.

The unusual anomaly was found at necropsy in a boy 3 days old. Extending from the apex of the left ventricle to within 1 cm. of the umbilicus was a funnel-shaped diverticulum in which the lumen was continuous with the cavity of the ventricle. Associated maldevelopments were: defective ventricular septum, hare-lip, cleft palate, acrania and fusion of the dura with the fetal membranes. The probable explanation of the cardiac anomaly offered is fusion of the region of the cardiac apex with the fetal membranes early in development, leading to traction and the formation of the diverticulum.

W. SAPHIR.

**MYXOFIBROMA OF THE ENDOCARDIUM.** M. D. ARIOL, *Virchows Arch. f. path. Anat.* **277**:501, 1930.

The report relates to a new-born infant who had manifested the clinical symptoms of cardiac failure during the two days that it lived. At necropsy there were found, on both the mitral and the tricuspid valves, tumor-like papillary outgrowths that extended into the cavity of the ventricles. Histologic examination revealed no evidence of fetal endocarditis. The outgrowths were the result of proliferation of the subendothelial stroma. They were developmental in origin, the process being one of localized excess formation of tissue.

W. SAPHIR.

**OBLITERATION OF THE PERIPHERAL BRANCHES OF THE PULMONARY ARTERY.** A. GOEDEL, *Virchows Arch. f. path. Anat.* **277**:507, 1930.

In two cases of hypertrophy and dilatation of the right side of the heart, there was obliteration of terminal branches of the pulmonary artery, the result probably of thrombosis or of embolism, with subsequent organization and recanalization. Arteriosclerosis, syphilis and nonspecific inflammation must also be taken into account in such obstruction of the pulmonary circulation. Obstructive lesions of the smaller pulmonary arteries may be the explanation of those cases of hypertrophy and dilatation of the right side of the heart in which one often finds only slight chronic bronchiolitis or slight moderate emphysema.

W. SAPHIR.

**THE ACTION OF INTRAVENOUSLY INTRODUCED COLLOIDAL SILVER.** H. KOLLER-AEBY and T. KOLLER, *Virchows Arch. f. path. Anat.* **278**:84, 1930.

The therapeutic intravenous administration of colloidal silver in infection has been employed since the procedure was proposed by Credé in 1895. Although the distribution of the deposited silver in experimental normal animals has been studied, there is little precise knowledge of the distribution of the silver and its probable mode of action in inflamed human tissues. This report is based on the histologic study of tissues removed in six fatal cases of infection, in which colloidal silver had been used, and of an acutely inflamed appendix from a patient who had received two intravenous injections of colloidal silver previous to appendectomy. In confirmation of the observations of others made on animals, deposition of silver in granular form was noted in the reticulo-endothelial system and especially in the Kupffer cells of the liver. The deposition of granular silver compounds was most marked in the inflamed tissues, and the intensity of the deposition

ran parallel with the severity of the local inflammatory process. The deposition is ascribed to local acidosis of the inflamed tissues, the silver being precipitated as inorganic salts. It is to the latter that colloidal silver owes such therapeutic action as it may have.

O. T. SCHULTZ.

**SYPHILITIC ULCER OF THE STOMACH.** G. L. DERMAN and M. A. KOPELOWITSCH, *Virchows Arch. f. path. Anat.* **278**:149, 1930.

For the clinician, syphilis of the stomach is not a rare disease. For the pathologist, it is extremely rare. The authors report an ulcerative lesion of the stomach that they believe to be syphilitic, and not merely a coincidental peptic ulcer in a person with syphilis. The patient, aged 31, died of lobar pneumonia following an acute hemorrhage of the stomach. Autopsy revealed, in addition to the pneumonia, syphilitic cirrhosis of the liver with multiple gummas and amyloid infiltration of the spleen and kidneys. In the prepyloric region of the stomach there was a saddle-shaped ulcer of the greater curvature. The floor of the ulcer, which was covered at the margins by a thin layer of mucosa, was formed by a greatly thickened submucosa with perivascular lymphocytic infiltration and obliterating endarteritis of the larger arteries. The granulomatous process extended down into the muscle coats.

O. T. SCHULTZ.

**DERMOID OF THE CEREBELLUM.** M. KORNFIELD, *Virchows Arch. f. path. Anat.* **278**:165, 1930.

The lesion described occurred in a woman, aged 33, who had suffered with headache since the age of 15 and with symptoms of increased intracranial pressure for two months previous to death. The tumor was the size of an almond and was situated in the pia of the inferior anterior surface of the vermis of the cerebellum. It had caused dilatation of the ventricular system. It contained small areas of bone, cholesterol crystals, hair follicles and hairs. Some of the latter had penetrated the cerebellum and had led to minute herniations of cerebellar tissue along the hairs. The tissue was the seat of inflammatory reaction, characterized by lymphocytic and plasma cell infiltration and by fibrosis. The lesion is ascribed to embryonic misplacement of epidermis. The author tabulates thirty-two examples of intracranial dermoid recorded from 1745 to 1927. He tabulates also twenty-one previously reported cases of epidermoid, dermoid and teratoid tumors of the cerebellum. Seven of these were dermoids.

O. T. SCHULTZ.

**CONGENITAL ATRESIA OF THE DUODENUM WITH DUPLICATION OF BILE AND PANCREATIC DUCTS,** K. KATZ, *Virchows Arch. f. path. Anat.* **278**:290, 1930.

Atresia of the duodenum in a child that died on the seventh day after birth was associated with duplication of the distal portion of the ductus choledochus and of the pancreatic duct. One bile duct and a pancreatic duct entered the duodenum above the atresia; the other pair, below the atresia.

O. T. SCHULTZ.

**CONGENITAL ATRESIA OF THE TRANSVERSE COLON.** H. POPPER, *Virchows Arch. f. path. Anat.* **278**:295, 1930.

Congenital stenosis and atresia of the intestinal tract occur most frequently in the anus and rectum and next most frequently in the duodenum. The condition is seen less often in the jejunum and lower end of the ileum and least frequently in the colon. In the case presented, that of a stillborn, full-term infant, the ascending colon was greatly enlarged and ended blindly. It was not normally attached to the lateral body wall, and the blind end lay in the left iliac fossa. The transverse colon was represented by a narrow cord 8 cm. long in the border of the transverse mesocolon. Microscopic examination revealed no intestinal tissue in this cord. The blind end of the distal colon was situated at the lower pole of the spleen and

corresponded to the splenic flexure. The rectum was also atretic. In his discussion of the mechanism of the conditions found, the author devotes chief attention to the enlargement of the blind ascending colon. The enlargement was not due entirely to simple mechanical distention, but in part at least to marked hypertrophy of the wall. The author believes that the anomaly described was the result of two simultaneous errors of development. One was hypertrophy and overgrowth of the ascending colon, similar to the process that leads to congenital megacolon of Hirschsprung. The other was an inhibition of development that led to atresia of the transverse colon and rectum.

O. T. SCHULTZ.

CONSTITUTIONAL VARIATIONS IN CRANIAL FORM AND OXYCEPHALY. H. GÜNTHER, *Virchows Arch. f. path. Anat.* **278**:309, 1930.

Günther presents measurements of a number of variations in cranial form that he believes to be dependent on constitutional factors, and discusses the variations in relation to oxycephaly, for which he prefers the name akrocranium. Oxycephaly, which is due to hypoplasia or retarded development of the base of the skull, is often merely a subjective impression. Substantiation of this impression requires deviation from normal cranial measurements and indexes of a definite order of magnitude. Most important are the measurements and indexes that express the relationship of cranial circumference to height. Two pure types of akrocranium are recognized, one with, the other without, exophthalmos and angular deformity of the maxilla. The cranial deformity is often associated with impairment of vision (akrocraniodyopia), increased fragility of the erythrocytes and hemolytic icterus (akrocraniodyshemia) and phalangeal abnormalities (akrocraniodysphalangia). Although the cranial deformity may be due to mechanical factors acting on the fetus after development has been initiated, in which case the deformity is an acquired one, in the majority of instances the deformity is the result of abnormalities in the genetic constitution of the germ plasm. The associated abnormalities Günther also believes to be constitutional in nature.

O. T. SCHULTZ.

THE EARLIEST TISSUE CHANGES IN ACUTE RHEUMATIC INFECTION. F. KLINGE, *Virchows Arch. f. path. Anat.* **278**:438, 1930.

Klinge describes, as the earliest tissue change in acute rheumatic fever, serous infiltration of the ground substance of connective tissue. This proceeds rapidly to fibrinoid degeneration and disorganization of the tissue. These changes have been noted in the capsule of the tonsil, the liver and the spleen, in the walls of blood vessels, in the synovial membranes and joint capsule, and in the connective tissue of the myocardium and of voluntary muscle. The exudative-degenerative reaction is followed by cellular infiltration of variable degree by lymphocytes and leukocytes. Cellular infiltration soon becomes associated with proliferation of the fixed tissues. The exudative-degenerative reaction and the cellular infiltrative-proliferative reaction are not two different types of reaction in acute rheumatic fever, but are successive stages of the changes that occur in the tissues. The exudative-degenerative reaction is the result of local tissue allergy.

O. T. SCHULTZ.

CONGENITAL MALDEVELOPMENTS: ATRESIA OF THE SMALL INTESTINE. G. BODON, *Virchows Arch. f. path. Anat.* **278**:529, 1930. VENTRAL HERNIA. Z. SZANTROCH, *ibid.*, p. 539. MALDEVELOPMENTS OF THE CEREBELLUM. H. TESSERAUX, *ibid.*, p. 555. COMPLETE AND UNILATERAL ABSENCE OF VERTEBRAL BODIES. A. FELLER and H. STERNBERG, *ibid.*, p. 566.

Four successive contributions are devoted to examples of congenital maldevelopments of the types indicated by the titles. Each reviews the theories that have been propounded in explanation of the genesis of the particular anomaly and attempts to interpret the latter in the light of these theories.

O. T. SCHULTZ.

SILICOSIS OF THE LUNG. H. BERGSTRAND, *Virchows Arch. f. path. Anat.* **278**:647, 1930.

The older literature on pneumoconiosis ascribed the fibrosis of this condition to the mechanical action of the inhaled particles. More recent studies of pulmonary silicosis ascribe a greater rôle to the physicochemical action of colloidal silicon than to the particulate character of the material. The greater degree of change caused in the lung by some silicious materials as compared with others may be due to the chemical composition. Since coal dust, stone dust and other inspired materials contain appreciable quantities of silicon, it is probable that the action of colloidal silicon is the primary factor in the various pneumoconioses. Bergstrand describes briefly the histologic changes in silicosis and correlates these with the roentgenologic changes in the first, second and third stages of the disease. The first stage is characterized by localized thickening of the alveolar septums, degeneration of elastic fibrils, localized emphysema and collagenous thickening of the interlobular septums. In the second stage, the lymphoid follicles of the lung are transformed into tubercle-like fibrotic nodules. In the third stage, there occur serous and fibrinous exudation into the alveoli, collapse of central portions of the lung and organization of the collapsed areas by fibrous tissue. The three stages represent merely quantitative differences in degree of the same process, not qualitatively different forms of tissue reaction.

O. T. SCHULTZ.

TUBEROUS SCLEROSIS. H. FERIZ, *Virchows Arch. f. path. Anat.* **278**:690, 1930.

Feriz devotes eighty pages to a detailed and richly illustrated description of a case of tuberous sclerosis that presented many interesting features. Chief among these was the fact that the patient, a woman, reached the age of 25 years, was psychically normal, and had never had any epileptiform seizures. Second in importance was the rare anomaly, crossed dystopia of the kidney. Removal of the fused single kidney, under the preoperative diagnosis of sarcoma of the left kidney, was followed by anuria and death. The malformed kidney was the site of a mixed tumor that contained areas of differentiated muscle, fat and angioma, areas of undifferentiated myosarcoma, liposarcoma and angiosarcoma, and still other areas in which the sarcoma tissue appeared to be undergoing transformation into carcinoma. The left vertebral artery arose from the arch of the aorta. Each retina contained several minute nodules that histologically were found to be gliomatous. There were a number of similar subependymal nodules. The tuberous areas of the brain contained no ganglion cells. Some consisted of differentiated excessive glia, still others of cellular gliomatous tissue, and in some the cellular tissue appeared to be undergoing transformation from sarcoma-like tissue to tissue of epithelial character. The patient had adenoma sebaceum of the face, a condition not infrequently associated with tuberous sclerosis. Feriz looks on tuberous sclerosis as primarily a congenital maldevelopment characterized by inhibition of development and of differentiation of the tissues of more than one organ system. The tissue, the development of which has been inhibited, may at some later period undergo blastomatous transformation.

O. T. SCHULTZ.

### Microbiology and Parasitology

COCCIDIOIDAL GRANULOMA. California Dept. Pub. Health, Special Bulletin 57, 1931.

This bulletin reviews the knowledge of coccidioidal granuloma as it occurs in California. A complete bibliography on coccidioidal granuloma is included. The early history of the disease in California is reviewed by Emmet Rixford, a pioneer worker on the disease. The etiology and symptomatology are discussed by Ernest C. Dickson; the diagnostic laboratory procedure and epidemiology, by N. Dorothy Beck.

THE RESISTANCE OF DEHYDRATED PNEUMOCOCCI TO CHEMICALS AND HEAT.  
F. P. GAY, K. N. ATKINS and M. HOLDEN, *J. Bact.* **22**:295, 1931.

*Pneumococcus* type I in the form of both virulent and avirulent ("smooth" and "rough") dissociants is susceptible when grown in broth to the usual disinfectants, heavy metals, dyestuffs, anhydrous solvents, phenol and iodine, and to certain more or less specific substances such as ethylhydrocupreine hydrochloride and bile salts. In two instances it could be shown (mercuric chloride, ethylhydrocupreine hydrochloride) that the "smooth" organism was more readily killed than the "rough" form. When the micro-organisms are collected by centrifugation and rapidly dried to constant weight over calcium chloride, a large proportion of the cells are killed, the surviving percentage depending on the technic employed. The surviving pneumococci may continue to decrease in number, but some, at all events, survive for as yet undetermined periods—for eighteen months at least. Desiccated but living pneumococci of both forms "R" and "S" are not killed in the absence of water by alcoholic solutions of the substances described, except in the case of the heavy metals (mercury salts, silver nitrate). Dried "S" pneumococci, contrary to the findings in moist cultures, are more resistant to mercuric chloride than the "R" forms. The thermal death point of moist "R" pneumococci (56 C.) is distinctly higher than that of moist "S" pneumococci. When the two dissociants are dried they both resist heating to 115 C. for thirty minutes, but are killed by exposure to temperatures of 120 C. and above.

AUTHORS' SUMMARY.

THE BEHAVIOR OF RICKETTSIA PROWAZEKI IN TISSUE CULTURES. H. PINKERTON and G. M. HASS, *J. Exper. Med.* **54**:307, 1931.

Typhus *Rickettsiae* are found in large numbers in sections of tissue cultures of scrotal sac exudate. Extensive multiplication of the organisms occurs, and new cells become infected. Organisms are seen in cells undergoing mitotic division. The organisms usually become less numerous after the sixth day in vitro, but in one instance organisms were extremely numerous on the sixteenth and twenty-first days. *Rickettsiae* in tissue cultures retain their intracellular location, even when infection is very heavy. They multiply exclusively in nonphagocytic cells, which are believed to be of mesothelial origin. Pleomorphism is much more pronounced in tissue cultures than in guinea-pig tissues, and is entirely comparable to that seen in the louse.

AUTHORS' SUMMARY.

TOXIC PROPERTIES OF FILTRATES OF HEMOLYTIC STAPHYLOCOCCUS AUREUS.  
J. T. PARKER WELD and A. GUNTHER, *J. Exper. Med.* **54**:315, 1931.

Sterile filtrates from certain hemotoxic strains of *Staphylococcus aureus* have several toxic properties, of which the most important are the hemotoxic, the necrotoxic, the leukocidic and the property of killing rapidly. The necrotoxic action appears to be caused by a constituent in the filtrates different from either the hemotoxic or the leukocidic one.

AUTHORS' SUMMARY.

SWINE INFLUENZA: EXPERIMENTAL TRANSMISSION AND PATHOLOGIC ANATOMY.  
R. E. SHOPE, *J. Exper. Med.* **54**:349, 1931.

Swine influenza has been induced in pigs by the intranasal instillation of material from spontaneous cases of the disease occurring epizootically in eastern Iowa. The experimental disease has the same features as the epizootic. It has been maintained for study by serial passages accomplished either by intranasal instillation or by pen contact. Eight strains of the virus have been established experimentally during three epizootic periods. The clinical disease induced by these eight strains has been, in general, the same, although its severity and mortality have varied. The principal features of the pathology of swine influenza are an



exudative bronchitis accompanied by marked damage of the bronchial epithelium and its cilia, a peribronchial round cell infiltration and massive pulmonary atelectasis. The latter is modified somewhat by a round cell infiltration of the alveolar walls. The lymph nodes, especially the cervical and mediastinal ones, are hyperplastic and edematous. There is usually a mild to moderate, acute splenic tumor. The mucosa of the stomach and colon is congested. The pneumonia following swine influenza is, characteristically, lobular in type and of the same general distribution as the atelectasis. The nonpneumonic areas of lung are extremely edematous and congested.

HEMOPHILIC BACILLUS FROM RESPIRATORY TRACT IN SWINE INFLUENZA.  
P. A. LEWIS and R. E. SHOPE, J. Exper. Med. **54**:361, 1931.

A hemophilic bacillus has been regularly obtained in culture from the respiratory tract of a series of swine experimentally infected with swine influenza and from a small number of spontaneous field cases of the disease. It has not been observed in respiratory tract cultures from a group of swine free from influenza. The cultural and morphologic characters of the organism have been described, and the name *Hemophilus influenzae* (variety suis) suggested. The organism exhibits marked serologic diversity, since only two of eight strains studied were serologically identical. It is usually nonpathogenic for rabbits and white rats, and irregularly pathogenic for white mice. One strain of the organism was pathogenic for guinea-pigs, while two others were not. Eleven of thirteen attempts to induce symptoms of disease in swine by intranasal inoculation with pure cultures of *H. influenzae*-suis were entirely negative. The remaining two attempts, which suggested a positive result, have been discussed. Attention has been called to the marked similarity that exists between nonindol-producing strains of *H. influenzae*-suis.

FILTRATION EXPERIMENTS AND ETIOLOGY IN SWINE INFLUENZA. R. E. SHOPE,  
J. Exper. Med. **54**:373, 1931.

It has been possible to demonstrate, in Berkefeld filtrates of infectious material from experimental cases of swine influenza, a virus that when administered intranasally to susceptible swine induced a mild, usually afebrile illness of short duration. The changes in the respiratory tract resembled those in swine influenza, but were usually much less extensive. When the filtrable virus was mixed with pure cultures of *H. influenzae*-suis and administered to swine a disease identical clinically and pathologically with swine influenza was induced. The data presented indicate that the filtrable viruses of swine influenza and *H. influenzae*-suis act in concert to produce swine influenza and that neither alone is capable of inducing the disease. One attack of swine influenza usually renders an animal immune to reinfection. Blood serum from an animal made immune in this way neutralizes infectious material from swine influenza in vitro, as shown by the failure of the mixture to produce disease in a susceptible animal. The virus can be stored in a dried state or in glycerol for several weeks at least. In one instance, dried material apparently retained both the virus and *H. influenzae*-suis in viable form for a period of fifty-four days. Fatal cases of experimental swine influenza have been observed in which *H. influenzae*-suis was the only organism that could be cultivated from the respiratory tract. Attention has been called to some features of marked similarity between epizootic swine influenza and epidemic influenza in man.

AUTHORS' SUMMARIES.

PROTEIN FRACTIONS OF A SCARLATINAL STRAIN OF STREPTOCOCCUS HEMOLYTICUS. M. HEIDELBERGER and F. E. KENDALL, J. Exper. Med. **54**:515, 1931.

A tentative method is described for extracting a labile nucleoprotein from scarlatinal *Streptococcus hemolyticus*. The product differs chemically and sero-

logically from the fractions prepared by subsequent alkaline extraction of the cell residues, and from protein obtained by the classic method for the extraction of bacterial "nucleoproteins." The new nucleoprotein is sensitive to weak alkalis and readily loses nucleic acid under these conditions. The protein degradation products resemble the alkaline-extracted protein fractions of the cell residues. The bearing of the properties of the new nucleoprotein on the chemistry of nucleoproteins in general is discussed, also the possible relation of the fractions obtained to the analysis of streptococcus antigens made by Lancefield.

AUTHORS' SUMMARY.

THE TRANSMISSION OF THE VIRUS OF MEXICAN TYPHUS FROM RAT TO RAT BY POLYPLAX SPINULOSUS. H. MOOSER, M. R. CASTANEDA and H. ZINSSER, J. Exper. Med. 54:567, 1931.

*Polyplax spinulosus*, the common rat louse, is easily infected with the virus of typhus by feeding on infected rats. As in the case of *Pediculus humanus*, such feedings are followed by the appearance of large numbers of *Rickettsia prowazeki* within the intestine of the insect. The virus of Mexican typhus can be transmitted from rat to rat by *Polyplax spinulosus* by methods of feeding simulating natural conditions. It seems, therefore, that this ectoparasite is an important factor in maintaining an endozoic of Mexican typhus among wild rats.

AUTHORS' SUMMARY.

THE RELATIONSHIP OF CERTAIN VARIANTS OF B. TYPHOSUS. F. B. GRINNELL, J. Exper. Med. 54:577, 1931.

The results of cross-agglutination and agglutinin absorption experiments with the motile smooth, nonmotile smooth, motile rough and nonmotile rough forms of *B. typhosus* are presented. Cross-agglutination between these four forms is complete, save that the motile rough antigen is under certain conditions only weakly agglutinated by the antisera prepared with the nonmotile forms. Cross-absorption of the somatic agglutinin of the four variants is complete, save that the motile smooth culture still shows granular agglutination with the anti-MS and anti-MR sera after absorption with these cultures. A theory of the antigenic composition of the four variants of *B. typhosus* is presented, based on the results obtained in these experiments. It would appear that, contrary to the usually accepted theory, the four variants have a common somatic agglutinin. To explain the difference between the smooth virulent forms and the rough non-virulent forms it has been assumed that the S forms contain a carbohydrate that is associated with virulence and which takes no part in the agglutination reaction.

AUTHOR'S SUMMARY.

AN ALBINO RAT COLONY FREE FROM MIDDLE EAR DISEASES. J. B. NELSON and J. W. GOWEN, J. Exper. Med. 54:629, 1931.

A special colony of albino rats was built up by selection and isolation from a population in which middle ear disease was highly prevalent. No cases of aural infection occurred in the selected group, whereas its predecessor showed a crude incidence of 57 per cent. The subjection of selected rats to a rachitic diet and to overcrowding did not predispose to the development of middle ear disease. The incidence of pneumonia was not similarly affected; thus, 52 per cent of the adult selected rats and 78 per cent of the adult stock rats showed pulmonary lesions. There was, however, a significant reduction in the number of cases that showed advanced pulmonary lesions. Certain theoretical considerations of middle ear disease and of pneumonia are discussed.

AUTHORS' SUMMARY.

ARTIFICIAL ACIDOSIS IN TRYPANOSOMA LEWISI INFECTIONS. R. W. LINTON and H. A. POINDEXTER, J. Exper. Med. **54**:669, 1931.

When the alkali reserve is artificially lowered in rats infected with *Trypanosoma lewisi*, the number of parasites in the blood is increased. The increase is large in the early stages of the disease, and becomes less marked as the number crisis is approached. Near the crisis, and after it, a lowered alkali reserve does not affect the number of trypanosomes. It has been shown that the observed increase does not result from a contraction of the capillaries of the inner organs, which would throw a large number of trypanosomes into the peripheral circulation, nor is the increase due to a greater reproductive activity on the part of the trypanosomes. The increase must, therefore, be due to an inhibition of the destructive forces of the host. It is suggested that the known production of organic acids by the pathogenic trypanosomes plays a similar rôle in inhibiting the destructive mechanism of the host, and is therefore of significance in the pathogenic activity of these organisms.

AUTHORS' SUMMARY.

IN VITRO TRANSFORMATION OF PNEUMOCOCCAL TYPES. M. H. DAWSON and R. H. P. SIA, J. Exper. Med. **54**:681 and 701, 1931.

Type-specific *S* pneumococci may be transformed from one specific *S* type into other specific *S* types entirely by in vitro methods. *R* forms of pneumococci, derived from *S* forms of one specific type, may be transformed into *S* forms of other specific types by the following in vitro procedure: the growth of small inocula of *R* forms in mediums containing vaccines prepared from heterologous *S* cultures. Transformation of type may be effected in this procedure by the use of small quantities of *S* vaccine, quantities representing the bacteria from as little as 0.1 cc. of the original culture. Transformation of type, as induced by this procedure, is most readily effected by employing anti-*R* serum in the culture medium. Transformation of type may be effected, however, in mediums that do not contain anti-*R* antibodies. Previous findings on the thermal characteristics of the property of *S* vaccines responsible for transformation of type have been confirmed and extended.

Further observations on the conditions under which transformation of pneumococcal types may be induced by in vitro procedures are presented. *R* cultures possessing only slight degrees of *R* stability are most suitable for transformation purposes by in vitro procedures. Vaccines prepared from cultures subjected to the action of bacterial enzymes liberated in old broth cultures, and during mechanical disruption of young bacterial cells, are not effective in inducing transformation of type. The property of an *S* vaccine responsible for transformation of type is not related to the specific soluble substance of pneumococcus. Attempts to effect transformation of type by the use of cell-free extracts of pneumococcus have so far proved unsuccessful.

AUTHORS' SUMMARIES.

CERTAIN MONILIAS PARASITIC ON MAN. R. W. BENHAM, J. Infect. Dis. **49**:183, 1931.

The parasite found in thrush, here referred to as *Monilia albicans*, is a well defined species which can be recognized and differentiated from related forms by its morphologic and cultural characteristics. *M. psilosis* of Ashford is apparently identical with *M. albicans* isolated from thrush. Many of the yeastlike organisms found in *erosio interdigitalis*, in chronic paronychias, in *perlèche*, in other dermatoses and in certain types of superficial glossitis should for the present be placed in this same species. Other species showing slight, but definite, morphologic differences are found on the skin and in sputum and feces. Thirty strains of *Monilia* were tested for virulence. Nineteen, identified as *M. albicans*, from various sources, were pathogenic for rabbits. Eleven strains of other types produced no lesions. Seven strains of *M. albicans* proved similar in antigenic content

as determined by the reciprocal absorption of agglutinins. The slight differences detected could not be correlated with the sources from which these strains were obtained. Agglutinative reactions, when used in conjunction with other methods, were found the most satisfactory means for the identification of these rudimentary forms.

AUTHOR'S SUMMARY.

THE INFLUENCE OF PUS AND BLOOD ON THE ACTION OF BACTERIOPHAGE.  
M. APPLEBAUM and W. J. MACNEAL, *J. Infect. Dis.* **49**:225, 1931.

Purulent exudate exerts a marked inhibitory influence on the lytic action of the antistaphylococcus bacteriophage, sufficient to explain the persistent survival of the bacteria in purulent collections within the body of a patient receiving treatment with potent bacteriophage. Even when diluted 1:1,000, a purulent exudate sometimes exerts a relative inhibitory effect in vitro. Heating the pus at 60 C. for thirty minutes diminishes this inhibitory effect only slightly. Similar dilutions of purulent exudate failed to show an analogous inhibitory influence on the lytic action of anticolon-bacillus bacteriophage. Undiluted citrated blood, undiluted defibrinated blood and diluted blood serum exercise an inhibitory influence on the antistaphylococcus bacteriophage, but there is considerable variation in behavior of different bacterial strains and apparently in behavior of different races of bacteriophage. Undiluted blood did not permit the multiplication of the colon bacillus under the experimental conditions employed, and the experiments with diluted serum failed to reveal a clearly evident inhibition of the anticolon-bacillus bacteriophage.

AUTHORS' SUMMARY.

INFECTION OF THE ACCESSORY SINUSES AND UPPER RESPIRATORY TRACT IN AVITAMINOSIS OF RATS. R. G. TURNER and E. R. LOEW, *J. Infect. Dis.* **49**:244, 1931.

The bacterial flora of the nasal cavities and middle ears of ninety-two albino rats is reported. A comparison is made of the bacteria encountered in these localities when they present suppurative lesions with the flora when suppuration is absent. *Staphylococcus aureus*, chromogen 6 and the colon bacillus were the outstanding pyogenic organisms encountered in the suppurative lesions. The incidence of chromogen 6 was greater in the suppurative, than in the nonsuppurative, loci in the xerophthalmic animals. The variations in the percentage of the staphylococci were relatively slight, while colon bacilli were encountered most abundantly. Environmental conditions apparently played a part in the incidence of colon bacilli and of staphylococci. The age of the animal, the previous storage of vitamin and the season had no marked effect on the bacterial flora. Environmental conditions were not responsible for the increased incidence of chromogen 6. Comparison of the three series investigated showed that this organism gained in percentage incidence with increased severity of the disease produced by the withdrawal of vitamin A. Experimental evidence is given to substantiate the alterability of this organism toward the Gram stain. Provisionally it remains to be classified as a chromogenic, gram-negative coccus falling in group 6 of Gordon's classification. Chromogen 6 apparently has an elective affinity for the mucous membrane of the upper respiratory tract and nasal cavities. It fails to thrive when injected intraperitoneally or intramuscularly into common laboratory animals. The pathogenicity of chromogen 6 is attributed largely to the lowered resistance of the mucous lining of the nasal cavities, brought about primarily through withdrawal of vitamin A from the diet.

AUTHORS' SUMMARY.

EFFECT OF BACTERIOPHAGE ON ANTHRAX IN WHITE MICE. P. B. COWLES and W. M. HALE, *J. Infect. Dis.* **49**:264, 1931.

These experiments are based on a special case—the use of bacteriophage in anthrax in white mice—but the nature of the factors present would seem to be

exceptionally well suited for definite results. The mouse is so highly susceptible to the disease that most of the controls succumbed to the almost surely fatal dose used. The strain of anthrax used was quickly and permanently lysed by the bacteriophage in high dilution. That no protection resulted from the administration of bacteriophage in several ways and that even contact between organisms and bacteriophage before injection usually failed to inactivate all of the former seem most significant in the present case, and may suggest the reasons for failure of bacteriophage therapy in other diseases. Under such experimental conditions bacteriophage simply does not seem able to act efficiently, if at all, in the tissues. That it was present and could be recovered even after death is all the more striking, although whether the principle recovered was some of the original material injected or whether it had developed very slowly in the body cannot be stated. It may be added that tentative experiments designed to show whether bacteriophage used as an antigen has any immunizing value against anthrax in mice, guinea-pigs and rabbits failed to demonstrate any protection. However, such variable factors as the amount of bacteriophage injected, the frequency of the injections and the time requisite for the development of immunity were not studied exhaustively, so that no final conclusion can be drawn.

AUTHORS' SUMMARY.

ELECTROPHORETIC STUDY OF STREPTOCOCCI OF SCARLET FEVER AND ERY-  
SIPELAS. L. E. SHINN, *J. Infect. Dis.* **49**:281, 1931.

The growth of the organisms of scarlet fever and erysipelas in a standard medium produce similar, but distinctly different, changes in the electronitrogen picture. The changes in buffering action produced by the growth of these organisms are different and show a relationship to the changes in the values of the migratory nitrogen. The toxin of scarlet fever alters its charge sharply between  $p_H$  7.03 and  $p_H$  7.38. It does not exist in the filtrates as a simple free substance.

AUTHOR'S CONCLUSIONS.

ERGOSTEROL CONTENT OF MYCOBACTERIUM. P. S. PRICKETT and O. N.  
MASSENGALE, *J. Infect. Dis.* **49**:297, 1931.

Nine cultures representing six species of *Mycobacterium* were found to contain no ergosterol when cultivated on 5 per cent glycerol nutrient agar. Unactivated ergosterol added to this medium was found to stimulate the growth and also the production of pigment by these cultures, whereas activated ergosterol showed no stimulation of the growth, but even a retarding of the growth in the higher concentrations used. The latter substance also stimulated the production of pigment. Pathogenic strains of *Mycobacterium tuberculosis*, both human and bovine, were more sensitive to activated ergosterol than the nonpathogenic strains of the same types.

AUTHORS' SUMMARY.

ACID-FAST MICRO-ORGANISMS. F. EBERSON and M. A. SWEENEY, *J. Infect. Dis.* **49**:303, 1931.

Cultivation in nonprotein mediums favored the loss of acid-fastness of a strain of tubercle bacilli. The strain was then avirulent and devoid of invasiveness in guinea-pigs and not allergic, and it failed to protect animals against a small dose of virulent culture. Lipoidal substances in culture mediums did not transform ordinary nonacid-fast bacteria into acid-fast types or alter the tinctorial characteristics of tubercle bacilli. The staining reaction of an acid-sensitive strain of tubercle bacilli was not affected by varying temperatures. Virulent acid-fast bacilli differed from avirulent acid-sensitive strains in their reaction to dyestuffs that constitute the reagents in the Ziehl-Neelsen acid-fast staining technic. Neutral fuchsin favored the development of acid-sensitive organisms in the avirulent strain. Tinctorial effects of other dyes seemed related inversely to the bactericidal action of given dilutions. Acid-fastness was a characteristic of organisms that resisted

the lethal action of concentrated solutions of the given dyes. The virulent strain H37 failed to grow in culture medium to which the same dyes had been added. The acid-fast variants in the avirulent strain of tubercle bacilli did not exhibit the same degree of sensitivity to environmental influences as the original acid-fast strain. A culture that had become acid-sensitive could not be readily converted into the original acid-fast type.

EDNA DELVES.

DOUBLE INFECTION BY THE BRUCELLA GROUP. C. F. JORDAN and I. H. BORTS, Pub. Health Rep. 46:2437, 1931.

A Mexican laborer, aged 30, left his native country in February, 1930, took sick during April in Missouri, and was treated for undulant fever in a hospital in Iowa for thirty-two weeks. The blood culture yielded two strains of *Br. melitensis*—varieties *melitensis* and *abortus*. The *melitensis* variety of *Brucella* infection was in all likelihood acquired in Mexico because: (1) with this one exception, all of the cases of undulant fever in Iowa, so far as is known, have been due to *Br. melitensis*, variety *abortus* or *suis*, variety *melitensis* not being endemic in Iowa; (2) *Br. melitensis*, variety *melitensis*, infection is known to be endemic in Mexico; (3) the patient had contact with and used dairy products from goats in Mexico, but not in the United States. The source of the *abortus* variety of organism is not clear. A double infection may have developed before the patient left Mexico, as he used milk in addition to caprine dairy products. On the other hand, it is possible that the bovine infection was superimposed after the patient's arrival in Iowa. Pasteurized milk was used, but several cases of undulant fever are known to have occurred in the same community, with dairy products as the probable source of infection, one other case occurring within the same period.

AUTHORS' SUMMARY.

EFFECT OF HEMOLYTIC STREPTOCOCCI AND THEIR PRODUCTS ON LEUKOCYTES. A. EVANS, Pub. Health Rep. 46:2539, 1931.

Leukocytes are injured by acid. If the injury is not too great, they may be restored to a healthy condition by bathing in blood serum. In filtrates of broth cultures of *Streptococcus scarlatinae* there is a trace of a substance toxic for leukocytes which can be detected by the bioscopic test, but not by the phagocytic test or by the deterioration of cells as shown in stained microscopic preparations. The addition of kidney tissue, blood serum or washed leukocytes to broth cultures does not increase the production of the leukocidic substance. On the other hand, the addition of washed erythrocytes to broth cultures definitely promotes its increase. The thermolability of the trace of leukocidic substance in filtrate of broth culture is the same as that of the more abundant leukocidic substance in filtrate of culture in broth plus erythrocytes. Presumably the two substances are identical. A specific neutralizing agent for the leukocidic substance could not be demonstrated in normal or immune serum. Two lines of evidence are offered to show that the leukocidic substance is identical with hemolysin; they differ in thermolability, and there is no correlation of toxicity for the two types of blood cells manifest by filtrates of cultures grown under varying conditions. The decrease of leukocidic substance in purified and concentrated skin toxin indicates that the leukocidic substance and skin toxin are not identical.

AUTHOR'S SUMMARY.

A NEW MEDIUM FOR DIFFERENTIATING FORMS OF DIPHTHERIA BACILLUS. J. ANDERSON, F. HAPPOLD, J. MCLEOD and J. THOMSON, J. Path. & Bact. 34:667, 1931.

There are two principal forms of the diphtheria bacillus. *B. diphtheriae-gravis* is associated with severe toxic cases of the disease. It grows with granular deposit and pellicle in broth, has a flattened lusterless colony of irregular outline and

actively ferments polysaccharides. *B. diphtheriae-mitis* is associated with milder cases of disease in which there may be extensive membrane formation without serious intoxication, and which is probably chiefly dangerous from laryngeal involvement and obstruction of breathing. This form grows with uniform turbidity on broth, has a convex, partly translucent and light-reflecting colony and does not ferment polysaccharides. In addition, there have been observed from 5 to 10 per cent of intermediate forms giving granular growth in broth but failing to ferment starch, etc. The strain Park Williams 8 falls into this class. So far as they have been observed, the characters of these different forms are fixed. A blood agar medium is described, prepared with slightly heated broth sterilized by infiltration, and containing 0.04 per cent potassium tellurite, which permits determination of the presence or absence of *B. diphtheriae* at sight in 90 per cent of cultures from throat swabs after from eighteen to twenty-four hours of incubation. It also gives with the gravest types of diphtheria, those associated with *B. diphtheriae-gravis*, a picture so characteristic that it is not likely to be confused with anything else.

AUTHORS' SUMMARY.

EXPERIMENTAL STUDIES ON LYMPHOGRANULOMATOSIS. P. FOULON and P. LESBRE, *Ann. d'anat. path.* 8:477, 1931.

The authors undertook to test Sternberg's assertion that Hodgkin's disease is of tuberculous origin. They used three methods of investigation: animal inoculation, cultures of tissues removed from patients, and biologic methods (Bordet-Wassermann, intradermal reaction, etc.). The results obtained show that "lymphogranulomatosis, at least in the cases observed by the authors, has nothing in common with tuberculosis."

B. M. FRIED.

MECHANISM OF EXPERIMENTAL TUBERCULOUS INFECTION. A. BOQUET, J. VALTIS and A. SAENZ, *Ann. Inst. Pasteur* 46:373, 1931.

A careful study of the pathogenesis with regard to the guinea-pig indicates that the tubercle bacilli enter by way of the lymphatics, then into the blood stream and thus spread to the viscera. The rate of diffusion varies with the method of inoculation and with the number and virulence of the organisms. In massive infection, the bacteremia appears after an hour and persists until death. Progress is much slower with lighter inocula. Certain tissues, such as the pulmonary and serous parenchyma, owing to the extent of the surfaces and to the multiplicity of potential foci, are more affected than subcutaneous or submucosal connective tissue, accounting for the gravity of pulmonary, pleural or peritoneal infection, as compared to subcutaneous or digestive infection. From the fact that the grass bacillus invades in much the same manner as the tubercle bacillus, except in degree, it is concluded that the fatty structure is more involved than the actual pathogenicity. Actual virulence depends more on the ability to multiply in the tissues. Thus, with a slight infection with virulent organisms, the bacteremia may be so delayed that immunity offsets the effects. Localized foci develop, and latent infections may occur, as seen in naturally infected men and animals.

FROM AUTHORS' CONCLUSIONS.

NATURAL INFECTION OF THE RABBIT WITH TUBERCULOSIS. E. COULAUD, *Ann. Inst. Pasteur* 46:424, 1931.

Tuberculosis in the rabbit is of frequent occurrence, but it is often a latent infection that may show a spontaneous cure. Such tuberculous animals may not respond to tuberculin tests. Infection appears to occur during natural exposure or association with tuberculous animals. Most of the lesions noted were pulmonary, at times massive and progressive, often small, tuberculous nodules.

M. S. MARSHALL.

**PATHOLOGIC ANATOMY OF RABBITS INOCULATED WITH BCG.** D. D. LOKHOFF and I. K. LEVITAN, *Ann. Inst. Pasteur* 47:45, 1931.

Forty animals were divided into three groups. The first received BCG by intravenous or intraperitoneal routes, the second received virulent organisms intravenously, and the third received human tubercle bacilli intravenously followed by BCG (intravenously or subcutaneously) after five or more weeks. The average survival of treated animals exceeded that of the controls by fifty-three days, and the incidence of grave lesions was greatest in the control animals. On the injection of BCG, the progress of tuberculous processes is appreciably moderated, varying in degree with the route of inoculation. The tissues show distinct differences. The lungs of treated animals show proliferation of the connective tissue around the tubercles, and islets of caseous pneumonia in the form of diffuse sclerosis, points lacking in controls animals. Cicatrization is complete, and calcification of the caseous masses seems to occur more frequently. The spleens of the animals used as controls were normal; in the treated animals this organ usually showed hypertrophy and hyperplasia and hypertrophy of the cellules, suggesting mobilization in the reticulo-endothelial system. Other lesions were not distinctive. A marked inflammatory response of the lesions, as follows after the injection of tuberculin, was not observed.

M. S. MARSHALL.

### Immunology

**NATURE OF ANAPHYLAXIS.** DESPLANQUES, SIMONNET and VERGE, *Ann. Inst. Pasteur* 47:332, 1931.

"The potency of horse serum in inducing the anaphylactic shock in the guinea-pig seems uniquely bound to the globulin fraction; the serin appears to be an inactive albumin from the point of view of anaphylaxis of this species."

**THE PRODUCTION OF ANTITOXIN ON THE ADDITION OF NONSPECIFIC SUBSTANCES TO THE ANTIGEN.** G. RAMON, *Ann. Inst. Pasteur* 47:339, 1931.

In the course of weekly tests of antidiphtheria serum from a number of horses, it was noted that certain animals suddenly produced serums of especially high titer. The explanation was found in the previously reported fact that abscesses had developed at the points of injection of the toxin. This increase in the production of antibodies could be provoked at will by introducing nonspecific material with the antigen (powdered tapioca and calcium chloride proved the most satisfactory). By this means, the production of antitoxin by all animals could be increased, and adequate production could be secured in exhausted animals provided they were in good general condition. Similar results were obtained in immunization against tetanus. The principle is being applied to man. Diphtheria anatoxin is combined with typhoid vaccine, mixed in equal parts, and injected in 1, 2 and 3 cc. amounts at fifteen day intervals, to increase the effectiveness of the former. The results are said to have been excellent.

A. F. DEGROAT.

**EVOLUTION OF DIPHTHERIA ANTIBODIES IN HORSES.** A. BESSEMANS, G. RAMON and F. DE POTTER, *Ann. Inst. Pasteur* 47:358, 1931.

Subcutaneous injections of either toxin or living diphtheria bacilli into horses lead only to the production of antitoxin. No agglutinins are noted. Intravenous or intraperitoneal injections of living bacilli cause a transitory production of antitoxin, but specific agglutinins appear in abundance. The quantity of complement is variable and is independent both of the method of immunization and of the agglutinin and antitoxin titers of the serum. Thus, the evolution of antibodies is the result, not only of the type of antigen used but also of the method of injection. Estimations of the degree of immunity to diphtheria may best be based solely on antitoxin titer. The authors hold that their data support the hypothesis of the multiplicity of antibodies.

A. F. DEGROAT.



B C G VACCINE. H. BUSCHMANN, *Ann. Inst. Pasteur* **47**:374, 1931.

Children vaccinated by mouth with B C G vaccine showed 76 per cent positive reactions to B C G tuberculin intracutaneously. Subcutaneous vaccination resulted in 86 per cent positive tests. When the Pirquet test was used, the figures were respectively 61 and 63 per cent. Neither change in virulence, nor dissociation, nor adverse reactions were observed in the study of the vaccine.

FROM THE AUTHOR'S CONCLUSIONS.

LESIONS IN TUBERCULOUS GUINEA-PIGS TREATED WITH B C G. LEVITAN and LOKHOFF, *Ann. Inst. Pasteur* **47**:484, 1931.

Gross and histologic examinations were made of 130 guinea-pigs, inoculated with B C G between the twenty-ninth and the one hundred and eighty-ninth days of tuberculous infection. Besides two control groups, 79 animals were given human tubercle bacilli, principally intraperitoneally, followed by 0.002 and 0.0002 mg. of B C G subcutaneously every fourteen days. Macroscopically, 20 showed grave, 39 average, and 20 few lesions, very much as in the control groups. Microscopically, a difference both in the frequency and in the degrees of fibrosis was noted in the bronchial lymph nodes. It is concluded that, once the infection has started, B C G has little effect on the development of tuberculosis, but that the animals survive on the average twice as long. The reasons are apparent only microscopically: caseous or purulent pneumonic forms are rare; in the lungs and in the liver an interstitial inflammation is frequent; the spleen shows a more intense reaction of the reticulo-endothelial system; sclerosis and calcification of the lymph nodes are regularly noted.

M. S. MARSHALL.

PARALYSIS FOLLOWING PROPHYLACTIC TREATMENT FOR RABIES. S. G. MOFTAH and M. S. NABIH, *Office international d'hygiène publique* **23**: 2007, 1931.

Over a period of twenty years, 27,060 persons received antirabic treatment. The few cases of paralysis that developed were benign. Then, within two years, there were several deaths. Thirteen cases of paralysis varied in severity from a slight facial palsy to a Landry syndrome. All the patients in this group recovered. One, a laboratory worker, had been vaccinated yearly without ill effects, and then suffered a generalized paralysis after two successive treatments. In eight cases, the symptoms were those of acute myelitis. This diagnosis was confirmed at autopsy in four. In one case the cervical and in three the lumbar, enlargement of the cord was involved. These segments were hyperemic and edematous, and microscopically there were degenerations of nerve cells and fibers with widespread perivascular mononuclear infiltration. No Negri bodies were found in the brain, and animal inoculations were negative. Because animal inoculations were negative, and because one patient had received the phenolated vaccine, the authors conclude that the paralysis was due neither to the fixed nor to the street virus. They can suppose only that the vaccine contains a toxin. According to Babès, the toxicity of fixed virus sometimes increases after passage through a guinea-pig. The deaths reported occurred, in fact, shortly after two such passages.

ALBERT F. DEGROAT.

IMMUNIZATION OF GUINEA-PIG SPLEEN AGAINST TETANUS TOXIN IN TISSUE CULTURES. M. TOYODA, *Arch. f. exper. Zellforsch.* **10**:463, 1931.

Cultures of the spleen kept for some time in a medium containing tetanus toxin acquire the quality to tolerate considerably higher doses of toxin than would be lethal to normal cells.

WILHELM C. HUEPER.

THE ALLERGIC REACTION IN TUBERCULOUS INFECTION: ITS ANATOMIC ASPECT. P. SCHWARTZ and R. BIELING, *Verhandl. d. deutsch. path. Gesellsch.* **26**:226, 1931.

Rabbits the testes of which were infected with human tubercle bacilli were three weeks later inoculated intravenously with bovine tubercle bacilli. A few of these animals died from shock in the first days after the reinfection. Anatomically, there were noted pulmonary edema, splenic tumor with extensive cellular necrosis and necrosis of hepatic cells. In the rabbits that survived the second infection for from two to ten days, parietal intravenous granulomas were observed in the walls of the veins in the lungs and liver. They resembled the nodules described by Siegmund in scarlet fever. In animals killed from four to ten days after reinfection, the heart muscle showed smaller and larger nodules that looked like Aschoff's bodies in rheumatism. Also in rabbits that died from a single intravenous injection of bovine tubercle bacilli, identical anaphylactic morphologic changes were noticed, namely, hepatic necrosis, acute splenic tumor and inflammatory pulmonary edema. These lesions are explained by a spontaneous hematogenous reinfection with tubercle bacilli in the sensitized organism.

C. ALEXANDER HELLWIG.

REACTION OF THE LYMPHOID TISSUES TO ACTIVE IMMUNIZATION. T. HELLMANN and G. WHITE, *Virchows Arch. f. path. Anat.* **278**:221, 1930.

A series of twenty-three rabbits, divided into four groups according to their ages, were actively immunized by intravenous injections of a killed strain of *Bacillus paratyphosus* B. From one to five injections were made in periods of from three to sixty-four days. The lymphoid tissues were removed and studied by a method previously described by Hellman, by which nine tenths of the lymphoid tissue of the animal is subjected to examination. The chief effect of the process of immunization was noted in the spleen. This organ was enlarged, the enlargement being due to well marked hyperplasia of the germinal or reaction centers of the lymphoid follicles and of the white pulp and, to a lesser degree, to hyperplasia of the red pulp. The rest of the lymphoid tissues participated in the reaction, but not to so great an extent as the spleen. Increase in the size and number of the reaction centers of the lymphoid follicles is due to reticulo-endothelial hyperplasia, the hyperplasia being the result of stimulation by the process of immunization. The lymphoid tissues, through their reticulo-endothelial components, play an important part in the development of the immune state.

O. T. SCHULTZ.

THE RELATIONSHIP BETWEEN BACTERICIDAL ACTION OF THE BLOOD AND COAGULATION-INHIBITING SUBSTANCES. JORG KOSCHATE, *Zentralbl. f. Bakt. (Abt. 1)* **118**:60, 1930.

The immediate dilution of blood in ten parts of a 4 per cent solution of sodium citrate gave the highest colony counts from patients whose blood contained staphylococci, streptococci, paratyphoid bacilli and typhoid bacilli. The citrate solution was distinctly superior to heparin, bile, broth, potassium oxalate or magnesium hydroxide. Comparative experiments with these substances. *Staphylococcus aureus* and *Streptococcus hemolyticus* also showed the least bactericidal effect in the citrate-blood-bacteria mixture. The author attributes the favorable effect to the better inhibition of blood-clotting.

PAUL R. CANNON.

THE EFFECT OF ORAL IMMUNIZATION ON THE FORMATION OF HUMORAL ANTIBODIES. R. PFEIFFER and H. LUBINSKI, *Zentralbl. f. Bakt. (Abt. 1)* **118**:152, 1930.

Oral administration to rabbits of killed cultures of cholera spirilla led to extremely minute formation of humoral antibodies, although intravenous injection

tions of similar dosages gave a large yield. The authors believe that the production of antibodies following oral administration may be due to the passage of antigen through small lesions in the intestinal mucosa. Nevertheless, the results of the parenteral administration were 75,000 times better than those of the enteral, as measured by concentration of humoral antibodies.

Even poorer results were obtained in human beings treated with typhoid bacilli, only traces of antibodies being found in the serum following oral administration of the antigen.

If the presence of bactericidal and agglutinating substances in the serum is considered the criterion of immunization, oral administration gives no results indicating immunization.

PAUL R. CANNON.

PASSIVE IMMUNIZATION WITH HERPES ANTISERUM. HELMUTH FREUND, Zentralbl. f. Bakt. (Abt. 1) **119**:20, 1930.

The author obtained antiserum for herpes by injecting a nonlethal dose of the virus intracorneally. Reinjections were made in the same or in the opposite eye. The serums of such animals contained viricidal properties to a titer of 1:25.

The intravenous injection of such serums protected rabbits against spontaneous encephalitis after corneal injection of the virus, but had no influence in healing an existent herpes encephalitis.

Guinea-pigs previously treated with several doses of the rabbit antiserum were almost completely protected against the herpetic infection produced intracutaneously. Normal rabbit serum had no such effect.

PAUL R. CANNON.

IMMUNOLOGIC RELATIONSHIPS BETWEEN HERPES AND VACCINIA. HELMUTH FREUND, Zentralbl. f. Bakt. (Abt. 1) **119**:25, 1930.

Freund treated guinea-pigs intraperitoneally on five or six successive days with about 1 cc. of herpes antiserum obtained from rabbits infected intracorneally on several occasions with the virus. The guinea-pigs were then given intracutaneously, together with controls, vaccine virus, all injections being made into the plantar skin. The vesicles that developed were smaller and less well filled and showed a sharper line of demarcation in the serum-treated animals than in the controls. They also dried up more quickly in the former animals. These experiments confirm those of Gildemeister and his collaborators.

PAUL R. CANNON.

A HISTAMINE-LIKE SUBSTANCE IN RYE POLLEN (*SECALE CEREALE*). C. E. BENJAMINS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:189, 1931.

Extracts of rye pollen had a histamine-like effect on uteri of guinea-pigs and cats tested with the Dale technic, producing contractions in previously sensitized and nonsensitized animals. The substance is thermostable; it is resistant to the action of digestive ferments; it passes a dialyzing cellophane membrane under pressure. In the skin of healthy persons, it produces reactions identical with those called forth by histamine, with which it has also in common the effect on the blood pressure and iris of cats and rabbits, and on the chromatophores of the frog's skin. The substance in question is physiologically identical with histamine. No such substance could be detected in the following Gramineae: *Holcus mollis*, *Festuca rubra*, *Cynosurus cristus*, *Alopecurus pratensis*, *Phleum pratense*, *Dactylis glomerata* and *Lolium perenne*.

I. DAVIDSOHN.

THE INFLUENCE OF EXHAUSTION ON ANTIBODY PRODUCTION AND ON THE COURSE OF INFECTION. E. FRIEDBERGER, O. ANDERSEN, C. CALLERIO and I. RUTCHKO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:225, 1931.

Running, leading to complete exhaustion, had no effect on the titer of complement in guinea-pigs, on the normal antishoop hemolysin in rabbits, or on the

development of antishoop hemolysin and of bacterial agglutinins in immunized rabbits. Exhaustion did not seem to influence the course and outcome of the disease in rats infected with El Tor.

I. DAVIDSOHN.

THE PURIFICATION OF DIPHTHERIA TOXIN AND ANATOXIN. I. A. TASMAN and A. B. F. A. PONDMAN, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**: 245, 1931.

A comparative review of the methods used for purification and concentration of diphtheria toxin and anatoxin, with selection of the "Alum-method," for which a detailed procedure is outlined. The end-product, which is a toxin and not a toxoid, is not as durable as the original solution.

I. DAVIDSOHN.

A LOCAL SKIN REACTION WITH EXTRACTS OF PROTEUS X19 (THE EXANTHIN REACTION). LUDWIG FLECK, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:282, 1931.

Guinea-pigs infected with *Rickettsia prowazeki* lost the normal cutaneous reactivity to the extracts after about five to ten days following the onset of the illness. This lack of reactivity was specific, as extracts of *Proteus vulgaris* called forth the normal response. In rabbits, the same behavior was observed but only after from four to six weeks following infection. In rabbits immunized with *Proteus* X19, the cutaneous reactivity disappeared after about four to six months of immunization; they showed a somewhat increased resistance to infection with *Rickettsia prowazeki*. Rabbits immunized with killed *Rickettsia prowazeki* lost their cutaneous reactivity to *Proteus* X19. The *Proteus* X19 extracts could not be neutralized with a homologous rabbit immune serum, while such neutralizations sometimes occurred with typhus fever convalescent serums of man and guinea-pigs. Cutaneous hypersensitivity and hyposensitivity could sometimes be passively transferred. A close relationship and possible identity of *Rickettsia prowazeki* and of *Proteus* X19 is suggested by the results of the experiment.

I. DAVIDSOHN.

THE WASSERMANN REACTION FOLLOWING IMMUNIZATION WITH SPIROCHAETA PALLIDA. M. P. BATUNIN and R. R. HÖLTZER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:326, 1931.

This problem, repeatedly attacked in recent years, was here approached with somewhat different technic: (a) using various routes for injections, (b) prolonging the period of immunization, and (c) injecting guinea-pigs with living spirochetes. The results in guinea-pigs were perfectly negative; their serum did not react with alcoholic extracts of the spirochetes or with the usual organ extracts. In man, serums were obtained that showed a very weak fixation of complement with alcoholic extracts of the spirochetes and only transient inhibition of hemolysis with the other extracts. The method of immunization and the quantity of the injected antigen had no bearing on the serum response.

I. DAVIDSOHN.

THE CHEMICAL NATURE OF SO-CALLED SYPHILIS ANTIGENS. ÖDÖN FISCHER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:344, 1931.

Treating cholesterol-free phosphatides of beef heart with aluminium hydroxide ( $Al[OH]_3$ ) and with calcium phosphate ( $Ca_3[PO_4]_2$ ) removed their ability to act as antigens in complement-fixation and precipitation tests for syphilis. The change was not due to a decrease in the contents of the phosphatides and fatty acids or to a change in their relative proportions. Addition of fatty acids or of cholesterol did not effect reactivation of the extracts. Kaolin and barium sulphate ( $BaSO_4$ ) did not influence the antigenic properties of phosphatides of beef heart or had only a very slight effect.

I. DAVIDSOHN.

ALCOHOLIC EXTRACTS OF BACTERIA IN THE FIXATION TEST FOR SYPHILIS. HERMANN DEBUS, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:373, 1931.

Lecithin increased the antigenic efficiency and removed the anticomplementary action of alcoholic extracts of tubercle bacilli in relation to homologous antiserum and syphilitic serum. Proper quantitative relations between the extracts and the lecithin and proper technic in diluting the mixture with saline solution were important. The antigenic efficiency approached, but did not equal, that of cholesterolized beef extract. Mixtures of lecithin and of alcoholic extracts of diphtheria bacilli and of colon bacilli fixed complement with syphilitic serum but less efficiently than extracts of tubercle bacilli.

I. DAVIDSOHN.

EVALUATION OF SCARLET FEVER ANTISERUMS BY BLANCHING. F. V. BORMANN and A. WOLFF-EISNER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:411, 1931.

By comparative tests with various dilutions of different serums on the same patients the conclusion is arrived at that there is a direct relation between the ability of the serum to produce blanching of the cutaneous eruption and its therapeutic efficiency. Besides the dilution of the serum, the intensity and duration of the local effect must also be considered. The blanching phenomenon is due to a local antitoxic effect. (See articles by same authors on the blanching phenomenon in *Ztschr. f. Kinderh.* **51**:550 and 560, 1931.)

I. DAVIDSOHN.

BIOLOGIC DIFFERENTIATION OF MEAT BOILED FOR A LONG TIME. HERMANN RODENBECK, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:482, 1931.

The antigen used for the preparation of the precipitating serum consisted of a mixture of chicken meat and an extract of the meat obtained by treating it with saline solution and subsequently with sodium hydroxide. An immune serum prepared with this antigen precipitated extracts of meat boiled for a long time, while the usual precipitating immune serums failed to do so. With the help of such a serum it was possible to establish the presence of chicken meat in the commercial preparations of chicken bouillon. Complement-fixing antibodies could not be demonstrated. An attempt to produce specific precipitating serums with meat extracts failed.

I. DAVIDSOHN.

THE THERMOSTABILITY OF ANTIBODIES. LEO OLITZKI, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:498, 1931.

Dilution of immune serums to 1:100 protected the antibodies against the effect of heat equally well as the treatment with Bayer 205, with glycerin and with certain buffers as suggested by L. Silber, and had a wider range of applications. Protein-free antibody solutions, prepared according to M. Frankel and L. Olitzki, showed a marked resistance to heat of a character similar to that of diluted immune serums; treatment with Bayer 205, glycerin, etc., did not further increase their thermoresistance. The destruction of antibodies by heat is due to two factors: (a) coagulation of the proteins (in concentrated serums)—the coagulated particles take down the antibodies with them—and (b) direct destruction by heat (in highly diluted serums). This is well exemplified by the following experiment: Some of the agglutinins in a typhoid immune serum diluted 1:10, which were lost by heating, reappeared following treatment with hydrochloric acid and pepsin. The latter liberated the antibody by digesting the coagulated protein particles. The antibody destroyed by heat in a 1:100 dilution of the immune serum could not be regained by the digestive action, its disappearance being here due to the direct action of the heat.

I. DAVIDSOHN.

THE IMMUNOLOGIC RESPONSE OF SYPHILITIC RABBITS TO LIPOIDS. LUDWIG PRÜSENER, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* **72**:515, 1931.

The frequently reported positive Wassermann reaction found for normal rabbits is due to an improper technic. With the author's technic the reaction was regularly negative. Rabbits were treated with cow's milk, goat's milk, a non-specific milk preparation, an aqueous extract of guinea-pig kidney, an extract of the spirochetes of syphilis, and an aqueous extract of syphilitic rabbit testicles. Some of these rabbits were previously infected with syphilis, and their positive Wassermann reaction became negative. None of them developed a true positive Wassermann reaction, i. e., the ability to fix complement in the presence of alcoholic tissue extracts commonly used as antigens in the Wassermann test. Treatment with a mixture of lecithin and hog serum led to the development of positive Wassermann and Meinicke reactions, the latter appearing earlier than the former. The reagins so produced did not disappear after injections of arsphenamine nor could they be removed from the serum by the Sachs method.

I. DAVIDSOHN.

### Tumors

SARCOMA OF THE SPLEEN. LOUIS FRANK, *Am. J. M. Sc.* **183**:77, 1932.

Here have been added to the literature reports of two cases of sarcoma of the spleen, both being cases of the lymphocytic type. Sarcoma of the spleen may simulate pernicious anemia in its symptomatology and hemocytology.

AUTHOR'S SUMMARY.

BENIGN TUMORS OF THE BRONCHUS. H. WESSLER and COLEMAN B. RABIN, *Am. J. M. Sc.* **183**:164, 1932.

Based on a study of seventeen cases, we have sought to define a clinical picture of benign tumors of the bronchus. Our study has been concerned mainly with adenoma, which appears to be the commonest type of tumor encountered clinically. The following facts are emphasized: Care must be exercised in the microscopic diagnosis of these tumors lest they be mistakenly regarded as malignant. Benign tumors of the bronchus probably have a long period of latency during which there may be no symptoms of bronchial obstruction or bronchial irritation. In a considerable percentage of the cases this period is characterized by repeated hemorrhages. Aside from the symptoms of bronchial obstruction and infection, pulmonary hemorrhage is a frequent symptom of adenoma of the bronchus. This bleeding has certain characteristics that may suggest the diagnosis. When stenosis of a bronchus with infection of a lung has occurred, the clinical picture may be confusing. These clinical pictures are described. The prognosis of benign tumors of the bronchus depends on the early discovery and removal of the tumor, which may lead to prompt cure. When secondary inflammatory changes have occurred in the lung, the outlook is not good. Evidence is adduced which indicates that polypoid adenomas may undergo malignant degeneration.

AUTHORS' SUMMARY.

MELANOMA OF THE MENINGES. N. C. FOOT and P. ZEEK, *Am. J. Path.* **7**:605, 1931.

Two cases of melanoma, presumably primary in the meninges, are described. In one of them, a small primary tumor was shown in the choroid plexus with a copious petechial metastasis throughout the meninges of the brain and cord; the other was striking because of the presence of two good-sized tumors in the meninges, with metastasis to the lungs, a very unusual event in the case of cerebral tumors. It is believed that the facts brought out in the study of these cases point strongly to the validity of Masson's argument as to the origin of melanoma

in the nervous system; these tumors had nothing to do with the skin, so far as we could ascertain, and they showed the presence of fibrils that could only with difficulty be interpreted as representing anything but fibrils in some way connected with peripheral nerves.

AUTHORS' SUMMARY.

ON THE SILVER IMPREGNATION OF MELANOTIC TUMORS. N. C. FOOT, *Am. J. Path.* 7:619, 1931.

The microscopic examination of material from normal skin and mucosa, a variety of nevi and several melanoblastomas by means of a thoroughly reliable silver impregnation seems to bear out Masson's theory as to the relationship of pigmented moles and their malignant relatives to the nervous system. That the "Merkel-Ranvier," or "nevus" cell, divisible into tactile cells of various sorts, and the cells of the nonmedullated nerve fascicula all represent various phases in the life of one cell type in these tumors seems fairly evident, if not proved. That these cells may take the form of scattered individuals, nests resembling Meissner corpuscles, melanoblasts or neurofibromatoid complexes seems clear. In malignant tumors, it is only natural to expect more or less atypical growth and anaplasia and a return to structures representing stages in the fetal development of the cell. Alveolar and gland-like structures seen in "melanocarcinomas" should not, therefore, prove to be a very disturbing note in the theory. The silver impregnation as a means of recognizing and classifying these tumors cannot be too highly recommended; it is simple in operation, and experiment proves that it can be successfully carried out at the first attempt. It is to be regretted that it cannot, as yet, be successfully applied to paraffin sections, but as experiments are now under way, this difficulty may be solved. The striking difference between the epidermal and the nevus cells, when impregnated by this method, is at once evident, the association of fibrils with the latter afford a reliable criterion that is immediately applicable. In closing, it would not be trite to reiterate Masson's warning to investigators along these lines: Please do not rely on old, wornout methods while endeavoring to check up on this theory, but use those that Masson has perfected, or the one herein described.

AUTHOR'S SUMMARY.

MASSIVE UNATTACHED RETROPERITONEAL TUMORS. G. H. HANSMANN and J. W. BUDD, *Am. J. Path.* 7:631, 1931.

Seventeen retroperitoneal tumors that were not attached to adult urogenital organs are reported. All the tumors were similar to tumors that arise in the adult urogenital organs. Studies of retroperitoneal tumors collected from the literature integrated with the material of this paper have shown that almost all tumors that occur in adult urogenital organs may occur free along the course of development of the urogenital apparatus. The concept that they arise from remnants of the urogenital apparatus is the most logical explanation of their histogenesis.

AUTHORS' SUMMARY.

PAPILLIFEROUS TUMORS OF THE THYROID GLAND AND OF ABERRANT THYROID TISSUE. A. R. MORITZ and F. BAYLESS, *Am. J. Path.* 7:675, 1931.

One hundred and two papilliferous tumors of the thyroid gland or of aberrant thyroid gland tissue were studied and classified. Twenty-eight of these were from the Institute of Pathology of Western Reserve University and seventy-four were studied in published case reports from other courses. The differential characteristics of the various types are tabulated. These characteristics were not invariably present, but were the most commonly present. The papilliferous character of adenomas and malignant adenomas did not distinguish them from the non-papilliferous forms of those tumors so far as growth was concerned, while the

papilliferous cystadenomas and cystadenocarcinomas constituted a group of tumors having peculiar and characteristic growth. The term "papilliferous" as applied to tumors of the thyroid gland is significant only in connection with papilliferous cystadenomas and papilliferous cystadenocarcinomas.

AUTHORS' SUMMARY.

MYELOMA WITH UNUSUAL AMYLOID DEPOSITION. B. H. PAIGE, *Am. J. Path.* 7:691, 1931.

A case of multiple myeloma is reported with an associated extensive amyloidosis. Of interest, both clinically and pathologically, are the huge tumor-like masses that resulted from the deposition of amyloid in the striated muscles and about the shoulder joints. Worthy of note, also, are its presence in the spleen, kidneys, suprarenal glands, gastro-intestinal tract, heart, pancreas, reproductive organs, sympathetic ganglions and adipose tissue and its absence from the parenchyma of the liver.

AUTHOR'S SUMMARY.

TUMORS OF THE ISLANDS OF LANGERHANS AND HYPOLYCEMIA. M. G. SMITH and M. G. SEIBEL, *Am. J. Path.* 7:726, 1931.

Pancreatic tumors producing hypoglycemia are composed largely of abnormal beta cells. In cases of hypoglycemia due to a pancreatic tumor of island tissue, the normal island cells are not overstimulated. From anatomic studies in these cases there is no evidence that the activity of the normal island cells is depressed, although clinical studies of the sugar tolerance suggest this in some cases. Adenomas resembling islands of Langerhans in their cellular arrangement, which give neither clinical nor anatomic evidence of functional activity, may occur. Adenomas of the islands of Langerhans are not rare; even those with clinical symptoms are surprisingly frequent.

AUTHORS' SUMMARY.

PLACENTOMA IN YOUNG RATS AFTER GONADAL STIMULATION. M. C. SHELES-NYAK, *Am. J. Physiol.* 98:387, 1931.

Fresh extracts of beef anterior pituitary lobe were injected daily into young rats, and after four or five days the uteri were stimulated by threading. The injections were continued, and the animals examined on the third to the fifth day after this operation. With great uniformity, they showed deciduomatous nodules. Controls, in which a commercial extract of the pituitary gland was used instead of the fresh extract, failed to show these results.

H. E. EGGERS.

THE INFLUENCE OF HEREDITY ON THE OCCURRENCE OF CANCER IN ANIMALS. H. G. WELLS, *Ann. Int. Med.* 4:676, 1931.

The study of animals has shown beyond any question of doubt that heredity plays a great rôle in determining not only whether animals will or will not develop cancer, but also what the type and location of the neoplasm will be. It has also been established that heredity may determine whether cancer will or will not arise from a fairly constant type of injury. As demonstrated by Loeb and others, the combination of the genetic background with the stimulation is the essential factor in the production of cancer. The observations of Slye would appear to indicate that susceptibility to cancer behaves as a mendelian recessive, and that resistance to cancer is dominant. The study of transplanted tumors in animals indicates that they obey laws different from those obeyed by the spontaneous tumors of these animals. Histologically, the spontaneous neoplasms of animals present the same characteristics of growth as similar neoplasms present in human beings.

WALTER M. SIMPSON.



HEREDITY OF CARCINOMA IN MAN. A. S. WARTHIN, *Ann. Int. Med.* 4:681, 1931.

There would appear to be in some human families a dominant inheritance of the cancer factor, while in other families this factor manifests itself as a recessive inheritance. The great variation in susceptibility found in different members of the same family may possibly be explained by the great complexity of the cancer character. It cannot be a single, simple mendelian character, but may consist of a combination of a large number of factors. It is uncertain that in man the inheritance of susceptibility to cancer is always mendelian. From the available evidence we are certain of two factors: constitutional susceptibility to neoplasm and local predisposition of the organ to cancer. The first determines that a man may develop cancer; the second determines the organ or tissue involved. An important fact in proof of the hereditary nature of susceptibility to cancer is the occurrence of symmetrical neoplasms in members of the same family. If constitutional predisposition and organic predisposition to cancer are necessary to the development of a cancer, we should expect to find in identical twins examples of neoplasms affecting the same organ and the same part of the organ. This is precisely what has been observed in a number of cases.

From a proper interpretation of the influence of heredity of cancer in man it is necessary to consider at least four hereditary factors: the normal constitution resistant to blastoma; the pathologic constitution susceptible to blastoma; the normal resistant organ or tissue make-up, and the pathologic organ with cancerous predisposition. Each of these factors must be composite; no one is a simple unit factor in the mendelian sense. Each one represents large and complex genes in which a hundred or a thousand subsidiary factors may enter, which may mendelize independently or in combination. The old conceptions of dominant and recessive have lost their original significance as far as the inheritance of neoplasm in man is concerned. The possibilities of inheritance in the almost endless combinations that may result, the effect of diluent or intensifying combinations, the occurrence of lethal factors and their combinations, the action of the extrinsic factors of the environment and other modifying factors make the problem of the inheritance of carcinoma in man one beyond mathematical computation or prediction. The conception of mendelism that led Maud Slye to regard the inheritable susceptibility to tumor as a simple recessive unit character is all too primitive. Characters that show a dominant inheritance in several generations may be so modified that they thereafter show a recessive inheritance. Theoretically, the laws of Mendel have added much to the understanding of heredity, but their practical application in human heredity is limited because of the complexity of the problem.

WALTER M. SIMPSON.

GLAND EXTRACTS IN EXPERIMENTAL CARCINOMA AND SARCOMA OF ALBINO RATS. O. M. GRUHZIT, *Ann. Int. Med.* 4:1589, 1931.

Albino rats inoculated with Flexner-Jobling carcinoma, when treated with different extracts of suprarenal cortex, both with high and low epinephrine content, showed neither delay nor regression in the growth of the tumors as compared with untreated tumor-bearing rats or those treated with a nonspecific protein extract of ox testis. Similar negative results were obtained with extracts of thymus. Extracts of suprarenal cortex, thymus, omental lipoid and ox testis neither inhibited growth nor caused regression of Jensen sarcoma in albino rats.

WALTER M. SIMPSON.

### Medicolegal Pathology

POISONING OR SUDDEN NATURAL DEATH? F. NEUREITER, *Beitr. z. gerichtl. Med.* 11:32, 1931.

Two apparently healthy men, 50 and 56 years old, respectively, entered a coffee house and ordered coffee and rolls. After one of them drank half a cup, he suddenly fell to the floor, dying instantly. His friend called for the ambulance.

About five or ten minutes later, the second man suddenly became pale and drawn, and fell over dead. The coffee and rolls were examined chemically, but no evidence of a poison was found. The autopsy in the first case revealed a marked occlusion of branches of the coronary arteries, with many myocardial scars and marked thinning of the posterior wall of the left ventricle. There was no evidence of poison either in the content of the stomach or in the other organs. The autopsy in the second case revealed a hypertrophied heart, with aneurysmal dilatation of the anterior wall of the left ventricle and marked calcification of the coronary arteries. In addition, many old fibrous scars were found in the myocardium. The mouths of both coronaries were encroached on. The ascending aorta showed syphilitic aortitis. The content of the stomach and the various organs in this case were also examined chemically and revealed no traces of poison. The author states that the peculiar coincidence of the sudden death of the two friends in such a short interval could at first give rise to the suspicion of homicide and suicide. The autopsies, however, revealed that the sudden death of both was due to natural causes.

O. SAPHIR.

A RARE CAUSE OF SUDDEN DEATH: PRIMARY SCLEROSIS OF THE PULMONARY ARTERY IN A 12 YEAR OLD GIRL. F. PUNTIGAM, Beitr. z. gerichtl. Med. 11:106, 1931.

Sudden death of a 12 year old girl was the reason for a special investigation. Autopsy revealed marked arteriosclerosis of the pulmonary artery, with many small, yellow, circumscribed intimal thickenings, especially pronounced in both main branches. These branches were dilated and in some portions covered by mural thrombi, which were so large as to narrow their lumina markedly. The main pulmonary artery and the smallest branches showed no changes. In the course of the right pulmonary artery, several saclike dilatations were found. These dilated areas were filled with thrombi. Histologically, each of the larger branches of the pulmonary arteries showed a markedly thickened intima, which in places was twice as thick as the media. It consisted of a new formation of loose connective tissue. The internal elastic membrane was split in places, and the boundary between the media and the intima was not marked. The media also revealed a splitting of the intima and a new formation of connective tissue, with deposits of lime salts. Occasionally, small round cell infiltrations were noted in the media. The adventitia showed a perivascular infiltration by round cells. Regressive changes were not found. The heart was somewhat larger than normal. The right ventricle was larger than the left, dilated, its wall measuring 10 mm. in thickness, while the left ventricle measured 7 or 8 mm. The myocardium was normal. The ductus arteriosus was closed. The coronary vessels showed no changes. The peritoneal cavity contained 1,000 cc. of clear liquid. The liver, spleen and kidneys revealed marked chronic passive hyperemia.

The history which was obtained after the autopsy revealed that the patient had otitis media when she was 9 months old. At the age of 3, there was noted for the first time a disease of the circulatory apparatus, which increased in later years. Cyanosis was always marked thereafter. There was no edema or anasarca until a half year before death. Bloody sputum was often observed. The opinion is expressed that the otitis media had produced chronic inflammation of the pulmonary artery, which primarily was hypoplastic.

O. SAPHIR.

# Society Transactions

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## BUFFALO PATHOLOGICAL SOCIETY

*Regular Meeting, Feb. 12, 1932*

KORNEL TERPLAN, *President, in the Chair*

### SYSTEMIC RETICULO-ENDOTHELIAL PROLIFERATIONS WITH TUMOR-LIKE FORMATIONS IN A CASE OF CHRONIC LYMPHATIC LEUKEMIA. J. LOESCH.

During the last few years several reports have been published dealing with the proliferation of the reticulo-endothelial cells in various pathologic conditions. An additional case of unusual systemic proliferation with tumor-like formations is described.

The patient, about 47 years of age, a seaman, was admitted to the Buffalo City Hospital on July 1, 1931. He complained of shortness of breath, edema of the extremities and a sensation of pressure in the left upper quadrant of the abdomen. From the past history it was learned that he had a positive Wassermann reaction in 1925 when he applied for a position as a food-handler; thereafter, vigorous antisyphilitic treatment was given until 1929, consisting of several courses of arsphenamine, bismuth and mercury and one malaria treatment of ten chills; all of this, however, was without any effect on the positive Wassermann reaction. In 1931, the patient noticed for the first time a mass in the left axilla. At the time of admission, on July 1, 1931, he presented general enlargement of all the lymph nodes; the spleen was palpable and extended a hand's breadth below the costal margin; the lower extremities were markedly edematous. The red blood cell count was 3,655,000; the hemoglobin was 56 per cent (Sahli). The white cell count was 760,000. A differential count showed 100 per cent lymphocytes; occasionally a cell much larger than a normal lymphocyte was seen, with three nucleoli. The diagnosis was: chronic lymphatic leukemia. The patient failed rapidly and died on July 13, 1931. The outstanding observations post mortem were: marked enlargement of all the lymph nodes, of the liver and of the spleen. The bone marrow was red. The spleen and liver exhibited whitish nodules ranging from 3 mm. to 1 cm. in diameter. On cross-section, some of the lymph nodes were reddish and some whitish. Histologic examination showed that two lesions were present. First, chronic lymphatic leukemia, and second, marked proliferation of the reticulo-endothelial cells, forming intercommunicating strands in the spleen, liver and bone marrow and large nodules in the first two organs. The lymph nodes exhibited lymphatic leukemic lesions and, between the latter, large cell proliferations, bringing about nearly complete compression of the lymphatic tissue. At various points in the aforementioned cell proliferations phagocytosis of granular debris was exhibited, also red cells with pigment formation. In none of the situations were transitions from the lymphatic leukemic cells to the described proliferating cells noted. The lesions were explained as having developed independently along with the chronic lymphatic leukemia.

### THE CEREBRAL RESPONSE TO OIL INJECTED INTO THE BRAIN SUBSTANCE IN RABBITS. C. R. TUTHILL and G. M. BECK.

Mineral oil, olive oil and rabbit fat were injected into the brain substance of seventeen rabbits. The animals were put to death from twenty-four hours to fifty-two days after the operations. The earliest reaction appeared to be a retrogressive change in the oligodendroglia bordering the wounds, causing their nuclei to assume the form seen in polymorphonuclear leukocytes. The astrocytes grew

rapidly during the first three days and completed the walling off of the wounds in seventeen days, with but few glia in both radiating and parallel formation. The wounds tended to close with connective tissue and capillary reaction. The microglia produced the same rapid proliferation of fat granule cells as in stab wounds. The transformation of adventitial wall cells to microglia could be traced by the proliferation of adventitial wall cells in the perivascular spaces around twenty-four hour wounds and by the migration of these long, slender cells to the edge of the perivascular nervous tissue. The early migrated adventitial wall cell was characterized by the same morphology as it showed when present in the perivascular space. In wounds from two to five days old, the cells at the edge of the perivascular nervous tissue had increased in size and showed fine, barely perceptible filaments at the poles and from the cytoplasm about the nucleus. Immature bipolar forms with fine processes were also found in the nervous tissue at the edge of the wounds after four days, but elongated cells without processes were seen only at the edge of the perivascular nervous tissue. In older wounds and in uninjured parts of the brain, fully mature tripolar and multipolar cells were found at the edge of the perivascular nervous tissue. Division of fully grown microglia was never seen nor were there found round cells coming from the walls of blood vessels and migrating into the nervous tissue.

A complete report will appear in the *Archives of Neurology and Psychiatry*.

#### MESENTERIC LYMPHADENECTASIS. JOSEPH M. HILL.

A case of mesenteric lymphadenectasis in a white man, aged 60 years, was reported.

Clinically, the patient exhibited the picture of cachexia with nausea, pain in the epigastrium and frequent bowel movements, but an excellent appetite; this condition had developed over a period of about four months. No diagnosis was made. The patient gradually grew weaker and finally became comatose and died.

Post mortem, all the mesenteric lymph nodes as well as the retroperitoneal lymph nodes were found involved. They exhibited, grossly, a spongy, finely cystic appearance and ranged in size up to 2 cm. in diameter. Microscopically, the cystic appearance differentiated itself into distended, tortuous, communicating lymph node sinuses. The contents consisted of a homogeneous, thick, oily material, which stained red with scharlach R stain, but which was not doubly refractive. Not only were the lymph node sinuses entirely filled and distended by this lipoid substance, but also the lymphatic capillaries and lacteals in the intestinal mucosa.

The cellular elements of these lymph nodes suggested generally a marked response of the reticulum in a proliferation of the reticulum cells with the frequent formation of giant cells. Plasma and mast cells were numerous. The architecture of the lymph nodule was entirely obliterated, leaving only lymphocytic cell collections mingled with the cells described. Most of the nodes showed some few polymorphonuclear leukocytes, but in a number of the particularly smaller ones near the pancreas, the polymorphonuclears were the dominant type of cell; adjacent lymph vessels were found plugged with leukocytes, and a bacterial stain of this tissue showed numerous gram-positive streptococci.

The lymphadenectasis was interpreted as the result of obstruction in the lymphatic system proximal to the lymph nodes, the exact location of which could not be determined.

The cachexia might be accounted for by this obstruction.

Death resulted from the terminal type of hypostatic pneumonia.

#### A CASE OF GENERALIZED FIBROSIS CYSTICA OF BONE ASSOCIATED WITH TUMOR OF A PARATHYROID GLAND. RAYMOND S. ROSEDALE.

A case of generalized fibrosis cystica of the bone associated with tumor of a parathyroid gland in a white woman, aged 50 years, was presented. The clinical symptoms were marked loss of weight, polyuria, constipation, weakness, fatiga-

bility, and pain over the knees and right tibia. Physical examination revealed a firm nodule in the posterior aspect of the right lower thyroid lobe, emaciation, and tenderness over the calcanea and the right tibia. X-ray pictures revealed cystic areas in the ribs, clavicle, scapulae, tibiae and mandible, vacuolization of the skull, and generalized loss of density of the bones. The blood calcium was 16.5 mg., and the phosphorus 2 mg. per hundred cubic centimeters of blood.

The bone lesion on biopsy showed only osteoid tissue with some vacuolization, many osteoclasts, and active osteoblasts around the margin of the osteoid tissue. There was considerable fibrous tissue interspersed throughout.

The parathyroid tumor on removal was found to measure 2 by 1 by 1 cm. It was yellow in color and firm in consistency, with some cystic areas. The histologic diagnosis was: adenomatous hypertrophy with cystic degeneration of a parathyroid gland.

Acute urinary suppression developed, and the patient died four days after operation. Autopsy was refused.

MASSIVE ATELECTASIS OF THE LUNG FOLLOWING BRONCHIAL OBSTRUCTION BY  
CALCIFIED TUBERCULOUS TRACHEOBRONCHIAL LYMPH NODES. S. SANES  
and WARREN S. SMITH.

An unusual case of massive atelectasis with induration of the entire right lung following complete obstruction of the right main bronchus by tuberculous calcified lymph nodes was presented. A white woman, 37 years of age, became ill when she was 22 years old with an acute condition of the right side of the chest. Examination at that time showed dulness and displacement of the heart to the right side. No fluid was obtained on aspiration. In the next fifteen years, the findings in the chest remained unaltered, and provoked the diagnosis of pulmonary tuberculosis with fibrous changes and adhesions, and pleurisy with effusion. On no occasion were tubercle bacilli found in the sputum. Except for a mild dyspnea, the patient suffered little change in her general health. Death was finally due to lobar pneumonia (pneumococcal) of the left lung.

Autopsy revealed complete obstruction of the right main bronchus throughout its whole course, due to complete calcification of the right lower and upper tracheo-bronchial lymph nodes following tuberculosis, marked collapse-induration of the whole right lung, with total adhesive pleuritis; dextroposition of the heart, compensatory hypertrophy and emphysema of the left upper lobe, an old tuberculous focus in the apex of the left upper lobe, a few tuberculous nodules in the left bronchopulmonary and tracheo-bronchial lymph nodes, lobar pneumonia with fibrinous pleuritis of the left lower lobe, a few gray tubercles in the liver and left kidney, a slight, firm splenic tumor and bilateral serous cystomas of the ovary.

It is intended to publish a complete report in the *American Review of Tuberculosis*.

PREGNANCY FOLLOWING PROLONGED USE OF RADIUM. LOUIS A. SIEGEL.

The case that I am presenting is of interest from the physiologic, pathologic and clinical aspects. The patient, aged 37, a gravida for the fourth time, gave birth to her last previous child in 1922. For the next five months following her delivery, she had profuse flowing spells, for which she was curetted with the insertion of radium sufficient to produce an amenorrhea for two years. The histologic diagnosis of the scrapings was simple glandular hyperplasia. From 1924 to the time of writing the patient menstruated irregularly, missing from one to two months.

In 1930, she became pregnant. The expected time of delivery was very uncertain, and on Jan. 22, 1931, it was decided, following an examination, to perform a cesarean section. The indication for this procedure was the condition of the cervix, which was found to be markedly contracted and hard. It was believed that the cervix was fibrosed to a point that would not permit sufficient dilatation to allow delivery through the birth canal. At the operation, when the

uterus was cut into, the muscle wall showed evidence of a marked increase of fibrous tissue. A supravaginal hysterectomy was performed and when the cervix was cut across, the fibrosis was very marked, which corroborated the preoperative clinical impression. Both ovaries were markedly shrunk and elongated. A normal, healthy baby, weighing 8 pounds and 8 ounces (3,856 Gm.), was delivered. The postoperative convalescence was uneventful, and at the time of writing the patient is perfectly well.

This case illustrates, first, the injudicious and incorrect use of radium. Secondly, it demonstrates the possibility of the development of normal ova after prolonged interference with this function. This is in accord with the work of Dr. Douglas P. Murphy of Philadelphia, who has shown the effects of the x-rays and radium on animals before, during and after conception. Thirdly, the specimen is of interest as the lower segment and upper part of the cervix show a marked increase in fibrous tissue distributed around each muscle bundle and between the fibers. With a control specimen which had not been subjected to radium, van Gieson sections show a marked increase in the fibrous tissue in the case reported. Fourthly, cesarean section, in all cases presenting similar clinical findings, is the procedure of choice, as shown by histologic examination of the specimen.

This case is being reported by courtesy of Dr. Francis C. Goldsborough.

AN UNUSUAL CASE OF SEVERE PYEMIC EMBOLIC CHOLECYSTITIS WITH  
MULTIPLE LESIONS IN THE GALLBLADDER LEADING TO PERFORATION AND  
LOCALIZED PERITONITIS. K. TERPLAN and S. SANES.

The gross specimen and microscopic slides were presented. A complete report will be published in this journal.

## Book Reviews

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**Cytology and Cellular Pathology of the Nervous System.** Edited by Wilder Penfield, Professor of Neurology and Neurosurgery, McGill University, Montreal, Canada. Volumes 1, 2 and 3. Cloth. Price, \$30 net. Pp. 1,280, with 886 illustrations, 15 in colors. New York: Paul B. Hoeber, Inc., 1932.

This is the third in a series of works on cytology issued by the same publisher, the earlier being the "Handbook on Microscopic Technique," edited by C. E. McClung, and "Special Cytology," edited by E. V. Cowdry, now in its second edition. The present work is dedicated to Ramón y Cajal, the great leader in neurocytology.

The volumes before us are the products of internationally cooperative authorship by twenty-six neurocytologists in Canada, France, Germany, Holland, Spain, Sweden and the United States. The result is a comprehensive exposition in about thirty sections or chapters of the results of modern methods of studying the minute structure of the nervous system, including blood vessels, membranes, nerve sheaths and other structures not necessarily derived from neuro-epithelium. Each of the authors has handled his subject or subjects as seemed best to him. Each section or chapter has its own analytic table of contents, independently numbered illustrations and bibliography. To review each section separately is not feasible, and the most that may be done is to indicate briefly the scope and nature of the contents.

The first two volumes deal with the normal cytology and the general pathologic cytology of the nervous system and related structures, but the complete microscopic anatomy peculiar to any definite disease is not described. The sections come in this order: the general character of the neuron (Cowdry); the principles of the development of the nervous system (Kappers, Amsterdam); sensory ganglions and cranial and spinal nerves, normal and pathologic (de Castro, Madrid); the histopathology of nerve cells (Bielschowsky, Berlin); sheaths of the peripheral nerves, nerve degeneration and regeneration (Nageotte, Paris); nerve endings (de Castro, Madrid); nerves of blood vessels, heart, meninges, digestive tract and urinary bladder (Stöhr, Jr., Bonn); neuroglia, normal and pathologic (Penfield); microglia (del Rio Hortega, Madrid); choroid plexus and ependyma (Agduhr, Uppsala); cerebrospinal blood vessels (Cobb); the meninges, with special reference to the cell covering of the leptomeninges (Weed); pineal gland (del Rio Hortega); the hypophysis (Bucy); retina, choroid and sclera (Arey); the optic nerve and papilla (Cone and MacMillan).

The third volume is devoted to neoplasms, malformation and hematogenous reactions. The sections are as follows: cellular types in primary tumors of the brain (Bailey); tumors of the sheaths of the nervous system (Penfield); primary tumors of the spinal cord and intradural filum terminale (Kernohan); tumors of the optic nerve (Verhoeff); tumors of the retina (Grinker); tumors of the choroid and allied tumors (Friedenwald); neuroblastic tumors of the sympathetic nervous system (Bielschowsky, Berlin); neural proliferations in the vermiform appendix (Masson); tumors of the hypophysis (Bailey); malformation in the central nervous system—tuberous sclerosis, amaurotic family idiocy, aplasia axialis extracorticalis congenita, encephalitis periaxialis diffusa (Globus); hydrocephalus and the atrophy of cerebral compression (Penfield and Elvidge); inflammatory cells in the central nervous system (Greenfield, London); the cells in the cerebrospinal fluid (Boyd).

As pointed out by the editor in his preface, multiple sclerosis, muscular atrophy and other diseases are not considered. In a future edition it may be possible, perhaps, to make the presentation more complete on the pathologic side.

The illustrations merit commendation. They are placed in the text except in the case of the section on the retina, choroid and sclera, which is provided with eighty-three figures on six plates.

New or at least unfamiliar words are not infrequent. As examples that should receive the attention of the medical lexicographer may be mentioned: allocortex, archicortex, clasmatodendrosis, hygrophora, hyperepiphysia, hypo-epiphysia, iso-cortex, lemnoblasts, neurocrinia, neocortex, oligoglia, pachygyria, paraphysis, pathoclis, pitiocyte, pyronephore, sterocilia, sympathoblastoma, sympathogonia, sympathogonioma.

The pages are numbered consecutively through the three volumes, and there is a general index at the end of the third volume. It would have been advantageous to have indicated on the back of each volume the pages it contains. Of course, this defect may be remedied easily by pasting on the back of volume 2 a slip of paper giving the pages within its covers.

The book will be an invaluable source of information and guidance to the student and to the investigator of normal and pathologic neurocytology. The pathologist will welcome especially the sections on tumors and the illuminating discussions of the new classifications. To quote the editor: "It [the book] presents such insight into the field of future advance as may be granted to those who are contributing seriously to contemporary study of the normal structure and pathological variations of the nervous system."

**Microscopic Slide Precipitation Tests for the Diagnosis and Exclusion of Syphilis.** By B. S. Kline. Price, \$2.50. Pp. 124. Baltimore: Williams & Wilkins Company, 1931.

This book is an illustration of how not to write a laboratory guide dealing with the procedure of only one of numerous tests for the laboratory diagnosis of syphilis and how not to publish a book intended for workers trained in the technological field of one of the most responsible branches of medical laboratory technology. Many controversial issues of importance are dealt with in positive, almost final terms, all favorable to the test of the author. Certain important theoretical phases of the work are dealt with in chapters of 1 or 1½ pages, hardly deserving the dignified name of a chapter. The volume is disproportionately illustrated, and the illustrations are wastefully large. The illustrations on pages 2, 36, 45, 48, 54, 60 and 63 may serve their purposes at an exhibition, but detract from the dignity of the book and reflect on the adequacy of the training of "serologists interested in practical" laboratory work, for whom, according to the author's preface, the volume was ostensibly written. Of the 124 pages (from cover to cover) constituting the volume, 40 are either blank or given to pictures; 12 are given to the preface, table of contents, list of illustrations, references and index, and about 10 are given to table compilations. Less than half of the book is given to solid print. The book, in short, is a hybrid between a grade school self-instructor in manual training and an attempt to give a scientific foundation to a mere modification or improvement of a preexisting serologic technical procedure.



## Books Received

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THE AETIOLOGY OF TUBERCULOSIS. By Dr. Robert Koch. A translation from the German of the original paper announcing the discovery of the tubercle bacillus, read before the Physiological Society in Berlin, March 24, 1882, and published in the *Berliner klinische Wochenschrift*, 1882, XIX, 221, specially prepared for *The American Review of Tuberculosis*, March, 1932, by Dr. and Mrs. Max Pinner. With an introduction by Dr. Allen K. Krause. Price, cloth, 50 cents. Pp. 48, with 8 illustrations. New York: National Tuberculosis Association, 1932.

NEOPLASMS OF DOMESTICATED ANIMALS. By William H. Feldman, D.V.M., M.D., Division of Experimental Surgery and Pathology, the Mayo Foundation, Rochester, Minn. With a foreword by Charles H. Mayo, M.D. Cloth. Price, \$6, net. Pp. 410, with 193 illustrations. Philadelphia, W. B. Saunders Company, 1932.

TECHNIQUES DE LABORATOIRE APPLIQUÉES AUX MALADIES DE LA DIGESTION ET DE LA NUTRITION. Par Labbe, Labbe et Nepveux. Price, 140 francs. Pp. 886. Paris: Masson & Cie, 1932.

A TEXTBOOK OF GENERAL BACTERIOLOGY. By Edwin O. Jordan, Ph.D., Professor of Bacteriology in the University of Chicago and in Rush Medical College. Price, \$10. Pp. 819, with illustrations. Edition 10, entirely reset. Philadelphia: W. B. Saunders Company, 1931.

METHODS AND PROBLEMS OF MEDICAL EDUCATION. Series 20, The Rockefeller Foundation, 61 Broadway, New York, 1932.

MASS UND ZAHL IN DER PATHOLOGIE. Von Professor Dr. Robert Roessle, Direktor des Pathologischen Instituts der Universität Berlin und Dr. Frédéric Roulet, Oberarzt am Pathologischen Institut der Universität Berlin. Price, paper covers, 16 marks; bound, 20 marks. Pp. 144. Berlin: Julius Springer, 1932.

THE LIFE OF EDWARD JENNER, M.D., F.R.S., NATURALIST AND DISCOVERER OF VACCINATION. By F. Dawtrey Drevitt. Price, \$2. Pp. 127, with portrait. New York: Longmans, Green & Co., 1931.

PRIMARY CARCINOMA OF THE LUNG: BRONCHIOGENIC CANCER: A CLINICAL AND PATHOLOGICAL STUDY. In two parts. By B. M. Fried, M.D., Peter Bent Brigham Hospital, Boston. Price, cloth, \$5. Pp. 247, with 95 illustrations. Baltimore: Williams & Wilkins Company, 1932.

MICROSCOPIC SLIDE PRECIPITATION TESTS FOR THE DIAGNOSIS AND EXCLUSION OF SYPHILIS. By B. S. Kline, M.D., Chief of Laboratories, Mount Sinai Hospital of Cleveland, Assistant Professor of Pathology, Western Reserve University. Price, \$2.50. Pp. 99. Baltimore: Williams & Wilkins Company, 1932.

HISTOPATHOLOGY OF THE CENTRAL NERVOUS SYSTEM. An Introduction by Means of Typical Microphotographs and a Short Text by Prof. Dr. L. Bouman (Utrecht) and Prof. Dr. S. T. Bok (Leiden). Price, 25 florins. Pp. 37, with 212 illustrations. Utrecht: A. Oosthoek's Publishing Co., 1932.

PHYSIOLOGY OF BACTERIA. By Otto Rahn, Professor of Bacteriology, Cornell University, Ithaca, N. Y. Price, cloth, \$6. Pp. 438, with 42 illustrations. Philadelphia: P. Blakiston's Son & Company, Inc., 1932.

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